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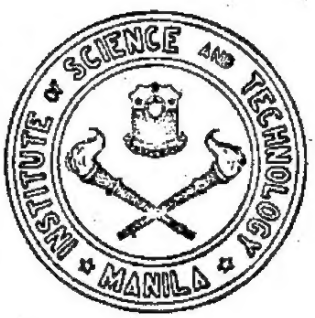

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PROCEDURE FOR EXTRACTION AND PARTIAL PURIFICATION OF ANTIMICROBIAL SUBSTANCES FROM ACACIA LEAVES

FRESH ACACIA LEAVES

Grind 100 gms Reflux for 2 hours in 400 cc distilled water or 95 per cent ethyl alcohol. Filter.

- **Residue**
- **Aqueous or alcoholic filtrate**

With alcoholic filtrate, distill to remove and recover alcohol. Restore original volume by adding water, Filter.

- **Aqueous filtrate**
  - Concentrate to 100 cc.
  - **Aqueous concentrate (1)**
    - Test a sample for antimicrobial activity → **Positive for inhibitory and growth-promoting substances**
    - Place in separatory funnel. Extract with petroleum ether.
    - Separate petroleum ether layer. Repeat extraction several times till petroleum ether is colorless. Combine all petroleum ether layers.
    - **Petroleum ether layer**
      - Concentrate till 1 cc represents 10 gms of leaves
      - **Petroleum ether concentrate**
        - Test a sample for antimicrobial activity
        - **Absence of inhibitory substance**
    - **Aqueous layer (2)**
      - Evaporate last trace of petroleum ether. Test a sample for antimicrobial activity → **Positive for inhibitory and growth-promoting substances**
      - Extract with chloroform. Separate chloroform layer. Repeat extraction till chloroform layer is colorless. Combine all chloroform layers.
      - **Chloroform layer**
        - Concentrate till 1 cc represents 10 gms of leaves.
        - **Chloroform concentrate**
          - Test a sample for antimicrobial activity
          - **Absence of inhibitory substance**
      - **Aqueous layer (3)**
        - Evaporate last trace of chloroform. Test a sample for antimicrobial activity → **Positive for inhibitory and growth-promoting substances**
        - Extract with ether. Separate ether layer. Repeat extraction till ether layer is colorless. Combine all ether layers.
        - **Ether layer**
          - Concentrate till 1 cc represents 10 gms of leaves.
          - **Ether concentrate**
            - Test a sample for antimicrobial activity.
            - **Absence of inhibitory substance**
        - **Aqueous layer (4)**
          - Evaporate last trace of ether. Test a sample for antimicrobial activity → **Positive for inhibitory and growth-promoting substances**
          - Evaporate to dryness. Extract with absolute alcohol. Filter. Repeat extraction three or four times with absolute alcohol. Combine all absolute-alcohol-soluble extracts.
          - **Substance insoluble in absolute alcohol**
            - Dissolve in water. Filter.
            - **Insoluble matter**
            - **Aqueous solution**
              - Concentrate till 1 cc represents 10 gms of crude leaves.
              - **Aqueous concentrate (6)**
                - Test a sample for antimicrobial activity
                - **Presence of growth-promoting and a trace of inhibitory substances**
          - **Alcohol extract**
            - Concentrate solution till 1 cc represents 10 gms of leaves.
            - **Alcohol concentrate (5)**
              - Test a sample for antimicrobial activity
              - **Presence of inhibitory and a trace of growth-promoting substances**

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DISTRIBUTION OF GLYCOSIDES IN THEVETIA PERUVIANA (PERS.) MERR. AND NERIUM INDICUM MILL.

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These two ornamental plants found throughout the Philippines in cultivation belong to the group of glycoside-yielding plants which are recognized for their cardiotonic properties. Therefore, they can be looked upon as promising substitutes for digitalis, an imported drug.

Thevetia peruviana (Pers.) Merr. commonly called *campanero* is described by Brown(2) as an erect, smooth shrub 2 to 5 meters high with elongated branches. The leaves are linear, stalkless, numerous and are 10 to 15 cm long and 7 to 10 mm wide. The corolla is yellow, funnel shaped and is 7 cm long and 5 cm broad. The fruit is a drupe, subglobose, smooth, green, shining, and is 3 to 4 cm in diameter.

Most of the investigators of this plant were concerned with the isolation and identification of the glycosidal material extracted from the kernel of the nut to which De Vry(7) first applied the name thevetin. The other researchers like Chen and his collaborators(4, 5) conducted pharmacological studies on the action of thevetin on frogs, cats, and other animals, while later Modell, et al,(10) published a report on the action of thevetin in man and compared its effects with those of digitalis.

Based on the review of the literature, relatively little attention has been directed to the distribution of this glycoside in the whole plant. However, only Pitchandi(11) reported that

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their Indian yellow oleander contains thevetin ranging from 0.38 to 0.48 per cent (bark, 0.38 per cent; root, 0.40 per cent; leaves, 0.41 per cent; and kernels, 0.48 per cent), obtained by following a colorimetric method based on the development of a stable pink color when thevetin is mixed and warmed with 60 per cent H_2SO_4. Casamada(3) stated that the leaves of *Thevetia* gives positive Baljet(1) color tests. It would therefore be of interest to find out if the Knudson and Dresbach(8) chemical method could be applied in the evaluation of *Thevetia* preparations.

The object of this investigation is to assay the extract of the different parts of this plant for its glycosidal content and compare the results with those of *Nerium indicum* Mill., locally known as *adelfa*, which the authors have reported previously.(6) Only the leaves and stems of three varieties of *Nerium* were studied, but in as much as two more varieties, the red and yellow, are now available, this work is being continued utilizing also the flowers of the five varieties.

Manalo(9) also applied the Baljet reaction to the alcoholic extract of the leaves and bark of two varieties of *Nerium* expressing their potencies not only in terms of ouabain equivalent but also in terms of digitalis units.

EXPERIMENTAL

The materials used in this investigation were the stems, leaves, flowers, fruit pulp, and kernel of *Thevetia* plants grown in Baliuag, Bulacan and Mandaluyong, Rizal. The former plant was very much older and bore larger fruits than the latter. In the case of *Nerium indicum* Mill., the flowers of the five varieties, namely, the deep rose pink, light pink, white, red, and yellow were collected and also the leaves and stems of the last 2 varieties.

All the tinctures were prepared by macerating 10-gm aliquots of the air-dried powdered parts of the plants with 100 cc each of 65 per cent alcohol with the exception of the dried kernels of *Thevetia* which were first defatted by treating with benzene. Shake for 3 hours, filter, and make filtrates measure 100 cc each. The marcs were then macerated again each with the same menstruum reduced to half the volume, this time making the filtrates measure 50 cc each. These tinctures prepared from first and second macerations were clarified and assayed separately following the Knudson and Dresbach method

with modifications. From the first maceration of the stems, leaves, flowers, fruit pulp and kernel of *Thevetia* 20, 15, 10, 20, and 6 cc respectively were clarified. In the second maceration 45 cc was used for all except the flowers which was 40 cc only.

Briefly, these tinctures were clarified by treating the specified volumes with 2.5 cc of 10 per cent neutral lead acetate solution to precipitate the tannins and diluting with water to 50 cc. Mix, fiter and the excess of lead in the 25 cc filtrate was removed by adding 1.25 cc of 10 per cent disodium phosphate solution, diluting to 50 cc with water, mix, and filter.

In the case of the first maceration of the stems, leaves, and flowers of *Nerium* 6, 5, and 4 cc, respectively were clarified while in the second maceration of same parts 40, 35, and 30 cc, respectively were used.

In the process of assaying, aliquots of these clarified tinctures were each mixed with 5 cc of alkaline picrate solution (4.75 cc of aqueous one per cent purified picric acid solution plus 0.25 cc of 10 per cent sodium hydroxide solution) made up to volume, and set aside for 20 minutes to develop the color. In our experiments, we used the Klett-Summerson photoelectric colorimeter with green filter No. 54 instead of the Dubosq colorimeter. Readings were then recorded and compared with 0.344 per cent aqueous solution of pure potassium dichromate used as a standard. Knudson and Dresbach claim that this solution gives a color equal in intensity to that of 0.5 cc of standard tincture of digitalis clarified and assayed in a similar manner. Since 1 cc of standard tincture of digitalis has a potency equal to 0.532 mg ouabain, therefore, the standard $K_2Cr_2O_7$ solution will give a colorimetric reading equivalent to 0.266 mg of ouabain. For computation the following formula was used:

$$\frac{0.266}{\text{Reading of standard}} \times \frac{\text{Reading of unknown}}{\text{Dilution of unknown}} = \text{Mg ouabain per cc of tincture}$$

Results are summarized in Tables 1 and 2. As can be seen from Table 1, it is interesting to note that when the alcoholic extracts of the different parts of the plant analyzed are compared, the glycosidal content–in terms of ouabain equivalent of the kernel (4.56 mg per cc) was found to be almost seven times as much as that present in the leaves, stems, or fruit pulp. This is followed by the alcoholic extract of the flowers which have an average of 1.59 mg per cc. The stems, leaves, and

TABLE 1.—*Potency of Thevetia tinctures in terms of ouabain equivalent.*

Source	Plant part	Tincture from first maceration		Tincture from second maceration		Total ouabain equivalent	
		Air-dried sample	Oven-dried sample	Air-dried sample	Oven-dried sample	Air-dried Sample	Oven-dried sample
		mg/cc	*mg/cc*	*mg/cc*	*mg/cc*	*mg/cc*	*mg/cc*
Baliwag, Bulacan	Stems	0.522	0.596	0.071	0.081	0.593	0.677
Do	Leaves	0.675	0.769	0.090	0.103	0.765	0.872
Do	Flowers	1.400	1.690	0.083	0.100	1.483	1.790
Do	Pulp (fruit)	0.578	0.532	0.098	0.108	0.676	0.740
Do	Kernel	5.084	5.110	0.315	0.323	5.399	5.433
Mandaluyong, Rizal	Stems	0.422	0.482	0.074	0.084	0.496	0.566
Do	Leaves	0.507	0.549	0.077	0.084	0.584	0.633
Do	Flowers	1.064	1.212	0.166	0.189	1.230	1.401
Do	Pulp (fruit)	0.590	0.618	0.054	0.056	0.644	0.674
Do	Kernel	2.68	3.47	0.168	0.216	2.848	3.686

fruit pulp contain approximately the same amount of glycoside which on the average is 0.69 mg per cc. This shows that the glycoside is not evenly distributed in the plant. The big difference in the amount of glycoside present in both kernels may be attributed to the difference in the age of the plant and size of the fruit.

On the other hand, the results summarized in Table 2 indicate that the glycoside is nearly evenly distributed in the case of the *Nerium* plant, for the 10 per cent tinctures prepared from the stems, leaves, and flowers of its five varieties have an average yield of 1.744, 2.247, and 2.670 mg ouabain equivalent per cc

TABLE 2.—*Potency of Nerium tinctures in terms of ouabain equivalent.*

Variety and plant part	Tincture from first maceration		Tincture from second maceration		Total ouabain equivalent	
	Air-dried sample	Oven-dried sample	Air-dried sample	Oven-dried sample	Air-dried sample	Oven-dried sample
	mg/cc	*mg/cc*	*mg/cc*	*mg/cc*	*mg/cc*	*mg/cc*
DEEP ROSE:						
Stems	1.108	1.227	0.123	0.136	1.231	1.363
Leaves	1.773	1.917	0.217	0.235	1.990	2.152
Flowers	1.900	1.990	0.190	0.199	2.090	2.189
LIGHT PINK:						
Stems	1.266	1.338	0.133	0.140	1.399	1.478
Leaves	1.934	2.029	0.281	0.294	2.215	2.323
Flowers	2.111	2.256	0.208	0.222	2.319	2.478
WHITE:						
Stems	1.477	1.501	0.147	0.152	1.624	1.653
Leaves	1.477	1.518	0.253	0.260	1.730	1.778
Flowers	2.556	2.806	0.221	0.242	2.777	3.048
Red:						
Stems	1.430	1.524	0.110	0.117	1.540	1.641
Leaves	1.834	1.980	0.253	0.273	2.087	2.253
Flowers	2.111	2.851	0.261	0.290	2.372	2.641
YELLOW:						
Stems	2.216	2.408	0.166	0.180	2.382	2.588
Leaves	2.128	2.472	0.223	0.259	2.351	2.731
Flowers	2.556	2.745	0.236	0.253	2.792	2.998

respectively. Comparing the different varieties of *Nerium*, our findings show that the leaves and stems of the yellow variety contain the most glycoside and those of the white variety the least, although the flowers of the latter have the highest. Basing on the total glycoside content of each variety, propagation of the yellow and red varieties should be encouraged for they have the most.

Nerium leaves and stems have three times as much glycoside as those of *Thevetia* although the kernel of the latter after extracting its oil amounting to 55.8 per cent contains the highest as stated before.

The glycosidal content of the tinctures from the second maceration of the different parts of *Nerium* and *Thevetia* ranges from $\frac{1}{7}$ to $\frac{1}{18}$ of that obtained from the first maceration so that a second maceration need not be performed, but instead the time of the first maceration with shaking should be increased.

SUMMARY

1. A comparative study of the distribution of glycosides in *Thevetia peruviana* (Pers.) Merr. and *Nerium indicum* Mill. both belonging to the Apocynaceæ family was made possible by the application of Knudson and Dresbach chemical method with modifications on the several tinctures (alcoholic extract 0.1 gm/cc) prepared from the different parts of the two plants.

2. A review of the results obtained in the two tables shows that the glycoside is nearly evently distributed in the *Nerium* plant (adelfa) while the opposite is true in the case of *Thevetia* plant (campanero). *Nerium* stems and leaves contain three times as much glycoside as those of *Thevetia* but the kernel of the latter gives very much higher yield than the parts of the *Nerium* plant examined.

3. Out of the five varieties of *Nerium* it appears that propagation of the yellow and red varieties is recommended because of their higher total glycoside content.

4. In the extraction of the glycosides with 65 per cent alcohol, the period of maceration with shaking should be increased in both plants.

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THE ANTIMICROBIAL ACTIVITY OF EXTRACTS FROM ACACIA LEAVES

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Acacia [*Samanea saman* (Jacq.) Merr.] is cultivated in the Philippines principally as a shade tree. Its pods, however, which taste sweet and which grow in great abundance during the dry season, are used as feed for livestock when there is scarcity of fodder. Local herbalists also used a decoction prepared from the inner tissues of its bark and from its fresh leaves in treating persons affected with diarrhea.(5)

A number of investigators have attempted to find more uses for acacia and what useful chemical substances could be derived from it. Padilla and Soliven(4) found that the kernel contains only 11.16 per cent oil, although it contain 59.72 per cent protein. Bhalerao and Dastur(1) analysed the pods for their nutritive value as animal feed; they found that they are a good source of protein, carbohydrates and minerals, and may equal good quality hay. Van Itallie(7) claims to have isolated from the bark two alkaloids (pithecolobine and a compound with a chemical formula of $C_8H_{18}NOCl$) and a saponin, and to have identified gallic acid, glucose, sucrose, fat, fatty acid, and a phytosterol. Weisner, et al.(8) studied the chemical structure of pithecolobine, but no definite use for the alkaloid and its derivatives has been reported by him. These investigations show that not much has been done along the medical uses of acacia.

Masiluñgan, et. al.(3) examined 339 Philippine plants for antibacterial substances. They reported that alcoholic and aqueous extracts from the leaves of acacia caused a small degree of inhibition of the growth of *Micrococcus aureus* and *Escherichia coli*. The senior author observed later that the same crude extracts possessed inhibitory activity against *Mycobacterium tuberculosis* 607 as shown by broad zones of inhibition of this organism around the filter-paper discs moistened with the extracts. Encouraged by the above results and by the fact that acacia trees grow in great abundance in the Philippines, the authors studied further the antimicrobial substances present in extracts from their leaves.

The purpose of the present investigation is to isolate the antimicrobial substances from acacia leaves and to determine whether the isolated and partially purified active substances have a wide spectrum of microbial inhibition by testing them against organisms represented by gram-positive, gram-negative and acid-fast bacteria and by a plant pathogenic fungus.

MATERIALS AND METHODS

The acacia leaves used in this investigation were obtained from trees growing within the premises of the Institute of Science and Technology, Manila.

Selection of solvents for extracting antimicrobial substances from the leaves of acacia.—The method of extracting the antibiotic substances from the acacia leaves and the technique of determining their effects on the growth of test organisms were essentially the same as those described by Masilungan, et al.(3) Water, ethyl alcohol, methanol, petroleum ether, chloroform, ether, and acetone were employed in extracting the antimicrobial substances from acacia leaves.

Test organisms.—*Micrococcus aureus, Sarcina lutea, Bacillus subtilis, Escherichia coli, Mycobacterium tuberculosis* 607, and *Fusarium moniliforme* were employed as test organisms. With the inclusion of *M. tuberculosis* 607 and *F. moniliforme* as test organisms, the nutrient media and method of seeding and incubating seeded media had to be modified. Glycerol agar and potato-dextrose agar were used respectively as test media. Incubation of test plate of agar seeded with *M. tuberculosis* was for 49 hours allowing one hour to lapse before placing the filter-paper disc moistened with the extract; that seeded with *F. moniliforme* was for 48 hours at room temperature, allowing 24 hours to lapse before placing the filter-paper disc moistened with the extract.

Methods of extracting the antimicrobial substances with the selected solvents.—Eight different processes of extracting the antimicrobial substances from fresh acacia leaves with preferred solvents (alcohol and water) were tried (Table 2). Each resulting extract was evaporated to a concentration such that one cc corresponded to one gram of the leaves.

Extraction and partial purification of the active antimicrobial substances.—After several preliminary trials for extracting and purifying the antimicrobial substances from acacia leaves, the procedure outlined below was finally developed. (Chart follow)

RESULTS AND DISCUSSION

Table 1 shows the diverse types of actions of the extracts toward the test microorganisms. As shown in this table, the different crude extracts were prepared by macerating acacia leaves in water, ethanol, methanol, petroleum ether, chloroform,

TABLE 1.—*Microbial-spectrum activity of extracts prepared by macerating ground acacia leaves in common solvents.*

Solvent-extract	Average diameter in millimeters of zone of inhibition or stimulation[1]					
	M. aureus	*S. lutea*	*B. subtilis*	*E. coli*	*M. tuberculosis 607*	*F. moniliforme*
Water	†††13.5	†††17.7	†††15.6	†13.5	††18.1	***15
Ethanol	††14.7	†††18.0	†††16.3	0	†††25.2	0
Methanol	††14.0	†††18.0	†††15.0	0	†††21.1	0
Petroleum ether	**14.0	**14.0	0	0	0	0
Chloroform	†††13.5 ***15.0	†††13.5 ***16.0	††15.0	††13.5	**13.5	**17.5
Ether	**16.0	0	**18.5	0	0	0
Acetone	0	††15.0	†††13.5	†13.5	†††22.0	0

[1] Inhibitive activity against test organism: ††† for complete inhibition; †† for partial inhibition; † for slight indication of inhibition; O for negative inhibition; Stimulatory activity: * * * for complete stimulation; * * for partial stimulation; * for slight indication of stimulation. The numbers refer to the diameter of the halo zone in millimeters. Example: † † † 13.5: * * * 15; means a complete inhibition zone of 13.5 mm; a stimulation zone of 1.5 mm beyond the 13.5 mm zone of inhibition.

ether, and acetone. The water extract inhibited the growth of all test bacteria used; but it stimulated the growth of a plant pathogenic fungus (*Fusarium moniliforme*). The effects of ethanol extracts were similar; both had none on the growth of *E. coli* and *F. moniliforme*, but strongly inhibited *M. tuberculosis* 607, *S. lutea* and *B. subtilis* and partially *M. aureus*. Petroleum ether and ether extracts did not inhibit the growth of any of the test organisms but they stimulated the growth of some of them, such as *M. aureus* and *S. lutea* in the case of petroleum ether and *M. aureus* and *S. lutea* in the case of petroleum ether and *M. aureus* and *B. subtilis* in the case of ether. Chloroform extract strongly inhibited *M. aureus, S. lutea* and *E. coli;* partially inhibited *B. subtilis* and *M. tuberculosis* 607; remarkably stimulated growth of *M. aureus* and *S. lutea;* and partially stimulated *F. moniliforme*. The acetone extract produced no effect on *M. aureus* and *F. moniliforme*, but partially inhibited

S. lutea, strongly inhibited *B. subtilis* and *M. tuberculosis* 607, and slightly inhibited *E. coli*.

Water, ethanol and methanol may be considered the best extractants of the antimicrobial substances, if the zones of inhibition of the test organisms caused by different crude extracts were compared. Owing to its high cost, methanol was not used in subsequent experiment.

The antimicrobial activities of the crude extracts prepared by different methods of extraction are presented in Table 2. Refluxing, decocting or macerating ground leaves in either alcohol

TABLE 2.—*Antimicrobial activity of extracts obtained by different methods of extraction.*

Method of extraction	Average diameter in millimeters of zone of inhibition or stimulation[2]					
	M. aureus	*S. lutea*	*B. subtilis*	*E. coli*	*M. tuberculosis 607*	*E. moniliforme*
Macerating ground leaves in alcohol for 24 hours	†††14.0 ***16.0	†††18.3 ***20.3	†††17.3 ***19.3	†††15.6 ***17.6	†††32.0	** 18.0
Macerating ground leaves in water for 24 hours	***14.0	†††17.6 ***20.6	†††16.6 ***18.6	†††15.3 ***17.3	†††29.6	**18.0
Infusing ground leaves in water for 1 hour	*15.0	†††16.0 ***19.0	†††16.3 ***19.3	†††13.5 ***15.5	††24.3	**17.0
Decocting ground leaves in water for 1 hour	*15.5	†††19.6 **22.6	†††16.0 ***18.5	†††15.6 *17.5	†††30.3	***17.0
Refluxing ground leaves in water for 1 hour	*15.0	†††15.0 **20.0	††††14.2 **19.3	†14.0 *17.0	†††29.5	***18.0
Refluxing ground leaves in alchool for 1 hour	†16.5	†††16.75 *19.75	†††16.25 **23.25	†16.0 *23.0	†††37.5	††25.0 **23.0
Percolating petroleum ether through ground leaves prior to refluxing in water for 1 hour	***15.5	†††17.6 ***21.6	†††19.0 ***20.6	†††16.6 ***18.6	†††29.0	*14.0
Percolating petroleum ether chloroform and ether successively through ground leaves prior to refluxing in water for 1 hour	***18.5	††17.0 *19.2	†††14.0 ***19.0	††14.0 **17.1	†††21.1	**21.0

[2] For explanation of signs and numbers in the above table, see footnote of Table 1.

or water extracted much of the active substance. An objection to macerating ground acacia leaves in water for 24 hours is the uncontrollable inroad of contaminants which interfere with the proper evaluation of the extract.

The extracts obtained by different processes were observed to be all inhibitory to *M. tuberculosis* 607; both inhibitory and stimulatory to *S. lutea*, *B. subtilis*, and *E. coli*; stimulatory to *F. moniliforme* and *M. aureus* except those obtained by refluxing in alcohol and those obtained by macerating in alcohol for 24

hours, they being both stimulatory and inhibitory respectively to *F. moniliforme* and *M. aureus*.

The crude extract obtained by refluxing in water, when tested against *F. moniliforme*, showed only growth-promotion. On further treatment of the same aqueous filtrate successively with petroleum ether, chloroform or ether, the partially purified aqueous layer, when tested for microbial-spectrum activity (Table 3), was observed to be both stimulatory and inhibitory to *F. moniliforme*. It was very apparent in this case that the predominance of the stimulatory activity over the inhibitory had prevented the latter from manifesting itself.

TABLE 3.—*Antimicrobial and growth-promoting activities of water-soluble[1] substances extracted from acacia leaves by refluxing in alcohol or in water.*[1]

Test organism	Refluxed in alcohol			Refluxed in water		
	Aqueous layer[2]	Alcohol-soluble concentrate[3]	Water-soluble concentrate from alcohol-insoluble residue[4]	Aqueous layer[2]	Alcohol-soluble concentrate[3]	Water-soluble concentrate from alcohol-insoluble residue[4]
M. aureus	†††16.8 **21.8	†††16.9 **18.0	0	**30.8	†††15.65 **20.15	***22.3
S. lutea	†††24.5 ***28.5	†††22.85 **23.0	†15.0 *19.0	†††24.15 ***30.65	†††24.25 **27.75	††17.1 *20.6
B. subtilis	†††25.3 ***27.8	†††22.55 ††24.2 ***26.85	†††14.0 **15.5	†††23.5 ††25.5 **27.5	†††24.6 ††26.6	†††17.2 ***22.2 **30.2
E. coli	††22.0 ***28.0	†††18.0 ††20.5 **21.9	0	†††17.5 ***23.3	†††19.3 **23.3	**22.0 **22.0
M. tuberculosis 607	†††41.0	†††40.25	†††14.0	†††43.5	†††44.15	†††15.5
F. moniliforme	***23.0 ††25.0	††27.0	***25.0	***22.3 †††24.3 ***28.3	†††18.0 **21.7 †††23.2 *20.0	***32.0

[1] For explanation of signs and numbers on this table see footnote of table 1.

[2] Refers to aqueous layer (4) of the procedure for extraction and partial purification of antimicrobial substances from acacia leaves.

[3] Refers to alcohol concentrate (5) of the procedure for extraction and partial purification of antimicrobial substances from acacia leaves.

[4] Refers to aqueous concentrate (6) of the procedure for extraction and partial purification of antimicrobial substances from acacia leaves.

Extracts obtained by refluxing in water for one hour, by percolating petroleum ether through ground leaves prior to refluxing in water for one hour and by percolating successively

petroleum ether, chloroform and ether through ground leaves prior to refluxing in water for one hour produced almost identical results (Table 2). The slight differences noted were in the size of zones of inhibition or stimulation of growth of the test organisms.

No remarkable difference in the inhibitory power of extracts obtained by different processes was noted except the fact that the one obtained by refluxing in alcohol always produced the biggest zone of inhibition of *M. tuberculosis* 607. In a further study of the antimicrobial activity of extracts partially purified by refluxing in alcohol and by refluxing in water the latter was found to possess higher inhibitory power against *M. tuberculosis* 607 than the former.

Decocting in water extracted slightly more of the active substance than refluxing in the same solvent, but the latter was finally adopted in subsequent experiment for convenience of the worker; the former required greater attention particularly in restoring periodically the water which was lost by evaporation.

The antimicrobial activities of the partially purified extracts as well as the other substances separated from them are presented in Table 3. The table reveals that partially purified extracts contain substances which are inhibitory and stimulatory to all test bacteria except *M. tuberculosis* which did not show any stimulation of growth after two days.[1]

The partially purified extract obtained by refluxing in water did not inhibit *M. aureus* but stimulated it instead. On further attempt to purify the same aqueous extract, the alcohol-soluble substances separated from it exhibited both inhibitory and stimulatory activity and the corresponding alcohol-insoluble substance which was soluble in water showed only stimulatory activity. This corroborated the preceding observation in the case of crude extract that the predominance of the stimulatory activity over the inhibitory, had prevented the latter to manifest itself.

Several attempts to separate the inhibitory substance from the stimulatory were made by treating the partially purified

[1] In an abstract entitled "The Antimicrobial Activity of Substances Extracted from the Leaves of Acacia (*Samanea saman*)" which was published in the Jour. Philip. Pharm. Assoc. 43 (1956) 186, this observation was included. It was found later by the senior author that prolonging the incubation of test plates to more than a week showed also the stimulatory effect of the extract on *M. tuberculosis* 607.

aqueous extract with absolute alcohol. There was an indication that it was possible since success in isolating pure antimicrobial substance specific for *B. subtilis* and *F. moniliforme* by treatment with absolute alcohol was obtained in two out of six trials.

As shown in Tables 1, 2, and 3 the extracts, in whatever way they are prepared, always contain substances which are highly antagonistic to *M. tuberculosis* 607. The antagonistic property of the purified active substance warrants further study. Work on purification and on chemical and physical properties of the active substances present in the leaves of acacia is in progress.

SUMMARY

1. The crude aqueous or alcoholic extract of leaves of acacia [*samanea saman* (Jacq.) Merr.] was observed to be antagonistic to a number of test bacteria affecting most prominently *Mycobacterium tuberculosis* 607.

2. Among the common solvents, namely, water, ethanol, methanol, petroleum ether, chloroform, ether, and acetone, used to extract the antimicrobial substances, it was found that water, ethanol and methanol were the most effective extractants.

3. Of the different methods of extracting the active principles from ground acacia leaves, refluxing in water or in alcohol, decocting in water, and macerating in alcohol gave satisfactory results.

4. Methods of extraction, isolation, and partial purification of the antimicrobial substances are described in the text.

5. Partially purified substances were tested and found to contain substances which were both inhibitory and stimulatory to *M. aureus*, *S. lutea*, *B. subtilis*, *E. coli*, and *F. moniliforme*. They produced broad zones of inhibition of *M. tuberculosis* 607.

6. The antimicrobial activity exhibited by partially purified substances, particularly against *M. tuberculosis* 607 warrants further study.

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STUDIES ON THIAMIN AND PROTEIN CONTENTS OF SOME LOCAL YEASTS

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Yeasts are the most widely known of all of the industrial microorganisms. Large amounts of yeasts are being produced yearly for use in baking, brewing and alcohol making. They are also used for nutritional purposes. In the United States the production of compressed yeast from industrial plants is reported by Frey(6) to be almost one-fourth billion pounds. This represents only about three-fifths of the entire yeast production of that country, two-fifths being made in the home.

In the Philippines the demand for yeast is also enormous. Statistics show that her yearly importation of yeast in the form of cakes, powder, tablets and pellets from the United States, France, and China during a 7-year period (1949-1955) averaged 318,344 kilos, valued at 604,416 pesos. Local production of yeast rose to 1,350,952 kilos, valued at 2,185,070 pesos, in 1955, lowering her importation in the same year to only 65,384 kilos, valued at 98,670 pesos. Local demand for yeast and yeast products still shows an upward trend.

The yeast cell is a minute factory where varied activities take place and where a large assortment of useful products, such as protein, glycogen, fat, vitamins, ergosterol and many other substances of yet obscure identity, are formed. It elaborates an array of enzymes which are ready to act on various organic substrates, and dissimilate them into valuable products, alcohol and glycerol being the best known of said products. The enzyme "invertase," which is formed by growing yeast, is now largely used by confectioners, bakers and syrup manufacturers in converting cane sugar into glucose and fructose by inversion.

The protein of the food yeast is said to be palatable and biologically complete, and is reported to be present in as high as 45 to 50 or more per cent of the dry product. According to Hock and Fink,(7) it is somewhat deficient in amino acid methionine and possibly in cystine; but, in spite of this, it is regarded as a valuable protein supplement. An appraisal

of "food yeast" based on its protein alone was made by Frey(6) who states that 10 million pounds of this product contain as much protein as ten thousand 1000-pound steers. These figures show that yeast offers a good source of high quality protein and that, therefore, under conditions of a national emergency the protein needs of the people may partially be met by producing it from yeast.

Yeasts contain the B-group of vitamins, such, as according to Prescott and Dunn,(8) thiamin, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, folic acid, and p-aminobenzoic acid.

The Philippines, being a tropical country, is naturally rich in microflora which include yeasts of numerous species, varieties and strains. An exploration of these microorganisms may lead to the discovery of species or strains which may be exploited both for industrial and nutritional purposes. This may be possible by the proper selection of the kind of yeast that would produce a large quantity of the desired product.

The purpose of the present investigation is to explore our yeast microflora for species or strains high in thiamin or protein.

COLLECTION OF YEASTS

Rotting sugary fruits, fermenting palm sap or *tuba*[1] fermenting vegetable leaves and starchy materials were collected. The presence of yeasts in these materials was determined by microscopic examination. The yeasts, if present, were isolated by pour-plate dilution method on Sabouraud's agar. Yeast colonies were readily distinguished, being usually white and somewhat slimy. Those showing irregular margin or wrinkled surface were observed to be formed usually by film-forming yeasts. Stock cultures on agar slants were made from single, isolated colonies.

COMPOSITION OF RAW MATERIALS USED AS SUBSTRATE FOR YEASTS

Cane molasses, corn kernels of the Yellow Flint variety, and mongo (*Phaseolus aureus*) beans were the important components of the substrate used for the propagation of various strains of yeasts used. Table 1 shows the analyses of the important constituents present in them.

[1] Tagalog term for the sweet sap obtained by tapping the young inflorescence of palms such as coconut, nipa, and buri.

TABLE 1.—*Chemical constituents of the raw materials.*

Constituents	Corn	Mongo	Cane Molasses
	Per cent	*Per cent*	*Per cent*
Moisture	12.36	10.20	19.49
Fat	4.83	1.42	----------
Protein	8.34	22.64	2.12
Carbohydrates	69.63	42.10	----------
Crude fiber	3.30	5.02	----------
Ash	1.52	3.68	8.37
Total sugar	----------	----------	61.63
Sucrose	----------	----------	35.22
Reducing sugars	----------	----------	26.41

SELECTION OF THIAMIN-PRODUCING YEAST STRAINS

Yeast strains in stock were each propagated in a molasses-salts medium [2] in flasks to obtain sufficient quantity of yeast cells for use in the detection of thiamin (Vitamin B_1). After incubating the culture for four days at room temperature, the yeast cells were collected by centrifuging and then dried in the oven at 50°C to constant weight.

A 10 per cent yeast extract was prepared by adding enough water to five grams of the powdered yeast to make a 50 cc mixture. A few drops of dilute hydrochloric acid were added to adjust the pH to 4.5. The mixture was heated gently for one hour at 70°C. After cooling, the original volume was restored by adding distilled water.

The presence of thiamin in the extract was determined by the thiochrome method described in the Pharmacopoeia of the United States of America(5) with slight modification as follows:

Ten cc of 10 per cent yeast extract was mixed with 5 cc of 0.5 N sodium hydroxide. To this mixture 0.5 cc of potassium ferricyanide test solution and 10 cc of isobutyl alcohol were added. The mixture was shaken vigorously to allow it to separate into two layers. any thiamin present in the extract was oxidized by the alkaline ferricyanide to thiochrome which was absorbed by the isobutyl alcohol layer. When irradiated

[2] Molasses-salts medium:

Molasses	81.0 gm
Ammonium sulfate	2.0 gm
Tri-sodium phosphate	3.0 gm
Sulfuric acid, conc.	20.0 gm
Water to make	1000.0 cc

Autoclave for 30 minutes at 10 pounds pressure; adjust to desired pH, after cooling.

with ultraviolet light through a fluorophotometer, Pfaltz and Bauer Model B, it fluoresced, emitting a blue light.

The yeasts that caused an emission of distinct sky-blue fluorescence were selected as potential sources of thiamin. Those that behaved otherwise were discarded. Of the 83 isolates tested, only seven were selected for use in the following studies.

THE SELECTED YEAST STRAINS

IDENTIFICATION

The yeasts, among the microorganisms, are difficult to study owing to the complexity of their structure and life cycle and to the confusion which still exists regarding their classification into genera and species. Their generic and species determination is a task for specialists. To have the selected thiamin-producing yeasts identified by a specialist, the senior author wrote to the Northern Utilization Research Branch of the Agricultural Service of the United States Department of Agriculture at Peoria, Illinois, U. S. A. Dr. Lynferd J. Wickerham, zymologist of the Fermentation Section, to whom her letter was referred, graciously identified the seven cultures labeled consecutively from Y–1 to Y–7 sent him. His identifications of the yeast which came in with his reply of July 10, 1956 are shown in Table 2.

TABLE 2.—*Origin and identification of the selected thiamin-producing yeast strains.*

Yeast culture	Origin	Name of organism [a]
Y–1	Rotting guava (*Psidium guajava*) fruit from Tanauan, Batangas	*Saccharomyces*
Y–2	Rotting papaya (*Carica papaya*) fruit from Pasay City, market	*Saccharomyces cerevisiae*
Y–3	Coconut *tuba* from Diot, Ilog, Occidental Negros	*Saccharomyces*
Y–4	Coconut *tuba* from Monasan, Ilog, Occidental Negros	*Saccharomyces*
Y–5	Nipa (*Nypha fruiticans* Wurmb.) "tuba" from Paombong, Bulacan	*Saccharomyces*
Y–6	Buri (*Corypha elata* Roxb.) "tuba" from San Jose, Batangas	*Candida guilliermondii*
Y–7	Rotting atis (*Anona squamosa* Linn.) fruit from Bocaue Bulacan	*Candida tropicalis*

[a] Genera and species are as determined by Dr. Lynferd J. Wickerham who believes that Y–1, Y–3, and Y–5 are all of the same species. According to him Y–1 and Y–3 produced the typical ascospores and had the fermentation reactions of *S. exiguus* and *S. chevalieri*. Y–4 and Y–5 did not produce ascospores but they had the fermentation reactions of *S. exiguus* and *S. chevalieri*.

FLASK CULTURES AT VARIOUS pH LEVELS

To determine the optimum pH for growth, each strain of the yeasts selected was propagated in molasses-salts medium of different pH levels as follows:

Three and one-tenth liters of molasses-salts medium was distributed in 100 cc portions into thirty 200 cc. Erlenmeyer flasks. After plugging, the flasks were autoclaved for 30 minutes at 15 pounds pressure and allowed to cool down to room temperature. They were then divided into batches of three flasks each. The different batches were adjusted to the pH levels shown in Table 3 and then each flask was inoculated with 2 cc of a water suspension of yeast cells from a 2-day

TABLE 3.—*Effect of pH on the dry product yield of seven selected yeast strains.*

pH	Dry yeast yield						
	Y-1 (Saccharomyces)	Y-2 (Saccharomyces cerevisiae)	Y-3 (Saccharomyces)	Y-4 (Saccharomyces)	Y-5 (Saccharomyces)	Y-6 (Candida guilliermondii)	Y-7 (Candida tropicalis)
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
3.7	0.2444	0.2247	0.2008	0.1912	0.1847	0.2560	0.2704
4.0	0.3981	0.3365	0.2541	0.2242	0.2245	0.2765	0.3725
4.5	0.4406	0.4333	0.3462	0.2846	0.2344	0.3862	0.4362
4.8	0.4114	0.3922	0.3267	0.2541	0.2672	0.4564	0.4993
5.0	0.3838	0.3764	0.3190	0.2442	0.2494	0.4308	0.4471
5.2	0.3568	0.3511	0.2977	0.2385	0.2351	0.4286	0.4259
5.5	0.3341	0.3409	0.2746	0.2276	0.2156	0.3998	0.3604
5.8	0.3264	0.3088	0.2489	0.2116	0.1989	0.3680	0.3585
6.0	0.2866	0.2466	0.2246	0.1987	0.1776	0.3254	0.3223
6.5	0.2364	0.2322	0.1998	0.1824	0.1670	0.2945	0.2637

old agar culture. After 5 days of incubation at room temperature the yeast cells in each flask were collected by centrifuging the culture, washed 4 times with water and then dried in the oven at 50°C to constant weight.

The average dry yeast yields of each strain at different pH levels are shown in Table 3. The figures in this table shows that the seven strains selected for this investigation have practically the same pH requirement. The optimum for all of them ranges from pH 4.5 to pH 4.8 This range lies within the requirement for optimum growth of *Torulopsis utilis* (Henneberg) Lodder which is placed at between 4.5 to 6.0 for some of its strains.(8, 9) It is close to the optimum pH for growth of the industrial yeasts *Saccharomyces cerevisiæ* of the brewing industry and *S. cerevisiæ* var. *ellipsoideus* of the alcohol or wine industry, and falls within the optimum range for some strains of these industrial organisms.(8)

THIAMIN CONTENTS

The thiamin content of the selected yeasts was determined by the thiochrome procedure which is described in "Methods of Vitamin Assay."(3) It is based on the oxidation conversion of thiamin to thiochrome which emits blue fluorescent light, and which under standard conditions and in the absence of interfering substances, produces fluorescence which is proportional to the thiochrome present and, hence, to thiamin. Fluorescence was measured with the aid of a fluorophotometer, Pfaltz and Bauer Model B. Calculation of thiamin was made according to the formula given in the same procedure.

Table 4 shows the thiamin contents of the local selected strains of yeasts which were propagated in molasses salts and corn-wort media at pH within the optimum range required for their growth. Of these yeasts Y–6 (*Candida guilliermondii*) leads in thiamin content, followed closely by Y–2 (*Saccharomyces cerevisiæ*) and Y–5 (*Saccharomyces* sp.). Y–1, Y–3, and Y–4 of genus *Saccharomyces* and Y–7 (*Candida tropicalis*) contain relatively much lower thiamin than the first three yeasts mentioned ahead.

The thiamin content of the yeast strains listed in Table 4 varies widely in the two different media in which they were

TABLE 4.—*Thiamin content of selected yeast strains in molasses-salts and corn-wort*[b] *media.*

Yeast strain	pH	Thiamin content per gram of dry yeast yield	
		Molasses-salt medium	Corn-wort medium
		Micrograms	*Micrograms*
Y–1 (*Saccharomyces*)	4.5	36.8	50.2
Y–2 (*Saccharomyces cerevisiae*)	4.5	41.4	73.7
Y–3 (*Saccharomyces*)	4.5	23.6	38.0
Y–4 (*Saccharomyces*)	4.5	24.5	40.1
Y–5 (*Saccharomyces*)	4.6	40.9	70.7
Y–6 (*Candida guilliermondii*)	4.8	51.2	84.8
Y–7 (*Candida tropicalis*)	4.8	34.8	47.3

[b] Preparation of corn-wort medium:

Ground corn	70.0 mg
Ground sprouted mongo seeds, previously dried at 50°C	52.5 gm
Ground sprouted corn kernels of the yellow flint variety, dried at 50°C	52.5 gm
HCl	0.5 cc
Water, sufficient to make	1000.0 cc

Cook ground corn in an autoclave at 15 pounds pressure for 30 minutes. Saccharify the cooked corn with a mixture of equal amounts of ground sprouted mongo seeds and ground sprouted corn kernels in 300 cc water until iodine test shows negative reaction for starch. Filter and wash with warm water. To the filtrate add water to make 1000 cc Autoclave at 15 pounds pressure for 30 minutes. Determine total reducing sugars by Munson and Walker gravimetric method.(2) Adjust the concentration of total reducing sugars to 5 per cent.

propagated. For the molasses-salts medium it varies from 23.6 to 51.2 micrograms per gram of dry yeast yield; for corn-wort medium it varies from 38.0 to 84.8 micrograms. From the point of view of thiamin production it is very apparent that corn-wort is superior to the molasses-salts.

Compared in thiamin contents with specifically known local strains of yeasts produced from the molasses-salts medium and with foreign strains propagated in more or less similar medium (Table 5), Y–6 (*C. guilliermondii*) is just as excellent a source of thiamin as the commercial Baker's yeasts and is better than many of the foreign or other local strains.

TABLE 5.—*Thiamin content of various strains of yeasts propagated in different kinds of molasses media.*

	Thiamin content per gram of dry yeast yield					
	Molasses-malt extract (beet)	Lansing (beet)	Mason City (beet)	Ovid (beet)	Hawaiian (cane blackstrap)	Molasses-salts (Philippine cane blackstrap)
	Micrograms	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*
Foreign:						
[As reported by Van Lanen, et al.(11)]						
Saccharomyces carlsbergensis	31					
S. cerevisiae (bottom yeast)	48					
S. cerevisiae (top yeast)	39					
Zygosaccharomyces pini	39					
Endomyces vernalis	34					
S. cerevisiae (Wildier strain)	29					
Torula utilis	29					
S. cerevisiae (distiller's strain)	47					
S. ellipsoides	38					
S. cerevisiae (Lash-Miller)	41					
S. cerevisiae A (Baker's yeast)	40					
S. cerevisiae B (Baker's yeast)	46					
S. cerevisiae C (Baker's yeast)	47					
[As reported by Agarwal, et al.(1)]						
S. cerevisiae		37.6	35.7	32.7	40.8	
Torulopsis utilis		37.5	36.7	38.1	35.4	
Candida arborea		32.7	33.1	31.3	33.1	
Oidium lactis		20.1	28.9	27.2	29.0	
Local:						
(As reported by Hipolito, et al.)						
Y–2 (*Saccharomyces cerevisiae*)						41.4
Y–6 (*Candida guilliermondii*)						51.2
Y–7 (*Candida tropicalis*)						34.8

This may be the first report of a thiamin-rich strain of *C. guilliermondii*. Other *Candida* species such as *C. arborea* and *C. tropicalis* (Table 5) have much lower thiamin content than this strain. *C. guilliermondia* (Syn. *C. guillermondii*) and *C. flareri* have been reported respectively by Burkholder(4) and Tanner and Van Lanen(10) to be a satisfactory source of

riboflavin but no mention of their being a good source of thiamin has been made.

The thiamin produced by yeasts propagated in the molasses media described above is considerably much lower than that produced by Baker's yeast from the commercial process. Baker's yeast with thiamin content of as much as 660 micrograms per gram of the dry yeast product has appeared in the market according to Van Lanen, et al.(11) They ascribe the increased thiamin content in such yeasts to the presence of pyrimidine and thiazole in the propagation medium. Experimenting on the synthesis of thiamin the same authors found that this vitamin is synthesized by the yeast when it is grown in a medium containing the pyrimidine and thiazole portions of the thiamin molecule and that "the efficiency of the conversion is 70 to 90 per cent provided the thiamin content of the yeast does not rise above 800 micrograms per gram."

PROTEIN CONTENTS

The different yeast strains were first propagated without aeration in molasses-salts and corn-wort media containing 5 per cent total reducing sugars with pH adjusted between 4.5 and 4.8. After 4 days incubation at room temperature the yeasts cells were separated by centrifuging, washed several times with water and then dried in the oven to constant weight. The protein contents of the dried yeast products were determined by the "Improved Kjeldahl Method" adopted by the Association of Official Agricultural Chemists.(2)

TABLE 6.—*Protein content based on dry yeast yield of the selected yeast strains propagated in molasses-salts and corn-wort media containing 5 per cent total reducing sugars.*

Yeast strain	pH	Molasses-salts medium	Corn-wort medium	Average
		Per cent	*Per cent*	*Per cent*
Y-1 (*Saccharomyces*)	4.5	46.60	46.78	46.69
Y-2 (*Saccharomyces cerevisiae*)	4.5	47.30	47.56	47.43
Y-3 (*Saccharomyces*)	4.5	45.30	45.48	45.39
Y-4 (*Saccharomyces*)	4.5	46.70	46.85	46.77
Y-5 (*Saccharomyces*)	4.6	45.50	45.78	45.64
Y-6 (*Candida guilliermondii*)	4.8	49.20	49.50	49.35
Y-7 (*Candida tropicalis*)	4.8	48.70	48.85	48.79

As shown in Table 6 there appears to be no striking difference in the protein content of the different strains whether

propagated in molasses-salts or in corn-wort media. Whatever difference there is, is shown to be in favor of Y–6 (*C. guilliermondii*) and Y–7 (*C. tropicalis*) with average protein content of 49.35 per cent and 48.79 per cent respectively. They have both made a slight edge upon Y–2 (*S. cerevisiae*), the yeasts having the highest protein content among the strains of *Saccharomyces* used in this study.

The protein contents of the *Candida* species mentioned above approximate that of *Candida arborea* which according to Agarwal, et al(1) contains 38.8 to 49.4 per cent and is one of the food yeasts that has been propagated commercially in Germany.(8, 9)

Having the highest protein content of all the strains used in this study, *C. guilliermondii* was further studied to determine if it possessed some more desirable characteristics by comparing its performance with the known food yeast *Torulopsis utilis* which was investigated by Yenko.(12) These two species were simultaneously propagated in molasses-salts medium in big battery jars to which air generated by ⅓-H. P. air pump and forced through 10-inch Mandler bacteriological filters at the rate of 1.35 liters per minute (112.5 cu cm per liter of medium per minute) was supplied. The required nutrient salts in the propagation medium were divided into 10 equal portions which were supplied at regular intervals during the 13-hour period of yeast propagation. The undesirable tendency of the medium to gradually become more acidic as growth progressed was obviated by hourly adjustment of its reaction to the desired pH with ammonium hydroxide. Excessive formation of foam was prevented by frequent stirring.

At the end of the propagation period the yeast crop was harvested by centrifuging the wort containing the yeast cells in suspension with the aid of Sharples Super-Centrifuge. The yeast harvest was washed and centrifuged alternately three times before determining the wet yeast yield and drying it to constant weight. The spent yeast-free wort was analyzed for residual reducing sugar and nitrogen and from the data obtained the amount of sugar and nitrogen assimilated was computed.

As shown in Table 7, *C. guilliermondii* has really the natural ability to synthesize in its cell as much protein as any food yeast, but it appears to lack one of the most essential charac-

TABLE 7.—*Comparative laboratory data on Candida guilliermondii and Torulopsis utilis from 6-run propagation each in 12 liters of aerated molasses-salts medium for 13 hours.*

Item	*Candida guilliermondii*		*Torulopsis utilis*
Total reducing sugars in medium, grams	179.71	299.53	179.37
Concentration of reducing sugars in medium, per cent	1.50	2.50	1.50
Total nitrogen in medium, grams	8.21	8.30	8.30
Amount of wet seed yeast used, grams	50.00	50.00	50.00
pH maintained at	4.80	4.80	5.00
Temperature of culture, °C	30–32	30–32	32–37
Gross yield of wet yeast, grams	170.00	300.00	500.00
Dry yeast yield, grams	39.10	69.00	100.00
Dry yeast yield based on total reducing sugars, per cent	24.19	26.79	55.76
Protein content of dry yeast yield, per cent	49.20	49.30	49.00
Assimilated sugars, grams	161.52	267.49	170.23
Assimilated sugars, per cent	89.88	85.97	95.50
Assimilated nitrogen, grams	3.07	5.44	7.84
Assimilated nitrogen, per cent	37.39	65.50	95.49
Alcohol produced, per cent	0.00	0.13	0.00

teristics of a commercial food yeast-rapid multiplication. Its yields in molasses-salts media containing 1.5 and 2.5 per cent reducing sugars are respectively 0.34 and 0.6 that of *T. utilis* indicating that it does not multiply as fast and as profusely as the latter under conditions in which the two organisms were cultured. This behavior may account for the lower percentage of nitrogen and carbon assimilation by this organism as shown in Table 7. The small amount of alcohol that it forms at sugar concentration of 2.5 per cent shows that complete aerobiosis of the medium is perhaps not attained.

T. utilis on the other hand grows rapidly and is able to utilize almost completely all of the nitrogen and carbon compounds supplied to the medium. These desirable features have not been duplicated by *C. guilliermondii*.

It is held possible that *C. guilliermondii* can still be made as prolific and as reproductive as *T. utilis* in molasses-salts medium by proper acclimatization to it and by understanding and supplying the essential factors for its optimum development.

SUMMARY

1. Eighty-three yeast isolates obtained from rotting fruits and fermenting vegetables, palm sap and starchy materials collected from several places in the Philippines were examined. Only seven isolates, five of which belong to genus *Saccharomyces* and two to genus *Candida* gave clearly positive reactions of the presence of thiamin and were, therefore, selected for further study not only for thiamin but also for protein contents.

2. The optimum pH requirement for growth and multiplication of the selected strains has been found to lie within the pH range necessary for the best development of food and alcohol yeasts.

3. Among the selected yeast, *Candida guilliermondii*, isolated from fermenting buri sap produces the highest amount of thiamin and can synthesize as much of this substance as the Baker's yeast or any other thiamin-producing yeast under similar conditions of propagation. The strain isolated from rotting fruits of atis and papaya and identified respectively as *Candida tropicalis* and *Saccharomyces cerevisiae* follow closely *C. guilliermondii* in thiamin cantent.

4. Minor variations in protein content are shown by the different strains. However, *C. guilliermondii* with an average protein content of 49.35 per cent leads all of them by a margin of 0.5 to 4.0 per cent. When this strain was compared with *Torulopsis utilis*, a well-known food yeast, it was found to lack some of the desirable characteristics of the latter such as rapid growth and ability to utilize all growth requirements from the cheap molasses-salts medium. By proper acclimatization to the raw material and supplying all of the missing factors favorable for its growth its yield may perhaps be increased.

ACKNOWLEDGMENT

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COMMERCIAL PRODUCTS FROM REFINED ALMACIGA

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Industrial trends in recent years have been towards the preparation of commodities entirely composed of, or containing substantial amounts of synthetic resins. In the absence of, or shortage of these synthetic requirements industry must rely upon other materials. One of these materials is natural resins. Concerning natural resins W. Krumbhaar [1] says:

> According to practical experience, certain valuable properties are more pronounced in natural gums, after they have been depolymerized, than in synthetic resins. The old time varnish makers used to cite a number of advantages of copal varnishes over varnishes based on synthetics. According to them, all copal ester varnishes have higher viscosity, better bodying and drying properties, greater toughness and durability of the dried film, than rosin ester varnishes, all other conditions being equal. Compared to modified phenolics, copals are reputed to produce glossier varnishes with less tendency to blooming. Copals are also considered to cause better adhesion of varnishes, especially when the latter are baked on metal; and they have the well established reputation of producing varnishes which, when used as interior can coating, do not give off any unpleasant odor or bitter taste to aqueous liquids, such as beer or fruit juices, with which they come in contact. When copal varnishes are compared to maleic resin varnishes, it is admitted that their dried films are dark in the beginning, but it must be emphasized that they soon bleach out; and it is stressed that copal varnishes, under otherwise equal conditions, are superior in rubbing and wearing characteristics.

In the Philippines the trade name for Manila copal is "almaciga."

One of the most prominent of the local natural resins now available in large quantities is almaciga. As a natural product Manila copal contains water or aqueous solutions in various proportions, considerable amounts of foreign matter such as sand, fragments of wood, bark, leaves, twigs and all kinds of organisms especially insects. An economical procedure for preparing a standard grade (refined almaciga) has been worked out. The refined product has a uniform composition and possesses all the desirable properties of the natural resin,

[1] Krumbhaar, W. Coating and Ink Resins (1947) 130.

completely free from extraneous matter and is entirely soluble in ethyl alcohol. It is this refined product that was used throughout the experiments.

Natural resins, besides, have a good many qualities that have not as yet been explored thoroughly, with the advent of new drying oils. Again, because of their reasonable price, they offer practical applications in the formulation of industrial preparations.

INSECTICIDAL BOOK VARNISH

(QUICK-DRYING)

Books deteriorate quicker in the tropics than in temperate countries because of the hot and moist atmosphere and of the prevalence of insects in the former. To preserve and lengthen their life, books should be protected from the effect of the tropical weather and the attack of insects. One way to keep them in good condition is by applying a good book varnish that is insect proof.

A good book varnish to serve as such should have certain qualities. It should be quick drying and should leave on the cover a durable film that does not stick. It should withstand normal wear or handling.

To have a book varnish that would approximate, if not meet completely, these requirements, a number of formulas were evolved and experimented on. Of the different formulas evolved, the following gave satisfactory results:

Formula

Refined almaciga	250 gm
Ethyl alcohol	1000 cc
Turpentine	120 cc
Castor oil	65 cc
Camphor	40 gm
DDT	10 gm

Preparation.—Place in a flask the refined almaciga, finely ground, in a portion of the alcohol (about 800 cc) and dissolve mixture by heating on a water bath with repeated shaking. Allow mixture to cool. And the castor oil previously dissolved in a portion of the turpentine. Dissolve the camphor crystals in

the remaining alcohol and add the solution to the mixture. Dissolve the insecticide in the rest of the turpentine and add lastly.

The container of the varnish should be well stoppered to prevent evaporation and affecting the concentration of the solution.

In treating books prefer a dry, sunny day, as the varnish does not dry well when applied to a moist and damp surface. In using the varnish, a small amount at a time should be poured out into another container.

The proper application of the varnish is also important. Moisten a piece of raw cotton with the liquid and apply slowly and carefully to give the book a smooth appearance. When dry, the book should be devoid of streaks and uneven spots. A second coating is advisable, to make a more uniform film.

WATER AND ALCOHOL RESISTANT VARNISH

For an all around varnish, the following has been found water and alcohol resistant, of the many formulas tried. This has qualities of waterproofness, hardness and durability of the film. It resists not only the effect of alcohol, hot and cold water, but gives a brilliant surface which does not scale or chip off.

Formula

Refined almaciga (run)	50 gm
Boiled lumbang or linseed oil	100 cc
Drier	20 gm
Turpentine	250 cc

Thermal processing (running).—Heat the resin slowly at first, gradually increasing the heat to about 315°C until the resin is melted. Continue heating for 2 hours at 315° to 325°C, until the foaming has subsided and the resin has turned into liquid showing a clean drip from the rod. This becomes hard on cooling. The resin is now soluble in oils.

Preparation of varnish.—Melt the run almaciga. Add the linseed oil which has been previously heated to about 288°C a little at a time with stirring. Continue heating for about 1.5 hours. A sample when cooled at this time becomes crystal clear. Allow batch to cool somewhat (232.2°C) and add the thinner. When cool, mix the drier. Allow to age before using.

LABEL VARNISH

In the Philippines, it is a common experience to see bottle and can labels destroyed by roaches. To protect these labels from these insects, the following formula of a label varnish which not only acts as a preservative but also enhances the gloss of the labels, has been evolved.

Formula

Refined almaciga	100 gm
Alcohol	300 cc
Castor oil	20 cc
Turpentine	50 cc
DDT	5 gm

Preparation.—Dissolve the almaciga in alcohol by heating on a water bath. Allow the solution to cool. Add the turpentine in which the castor oil and DDT have been dissolved and evaporate the whole mixture to a syrupy consistency by means of the water bath.

ORDINARY SEALING WAX

An ordinary sealing wax that is useful for sealing valuable papers and packages for storage or shipment may be obtained using the following formula. The primary constituent of this wax is plasticized resin with a pigment. In the form of sticks, heated over a direct flame, they may be applied on almost all kinds of papers.

Formula

Refined almaciga	45	gm
Rosin	15	gm
Turpentine	20	cc
Pigment	5	gm

Preparation.—Melt the resins first in a container large enough to permit quick stirring with a temperature maintained sufficiently low but enough to keep the material in a fluid state. Add the pigment previously made into a paste with the turpentine. Test the hardness and fracture of the mixture by dropping a small mass of it on a smooth cold plate. (All ingredients should be dry before using)

Molding.—Pour mass into a mold, which may be rectangular or round and made of a plain galvanized iron sheet bent to the desired size and shape. To facilitate removal of the finished product, provide both ends of mold with detachable wood stoppers.

STICKY FLY-PAPER

A fly-paper that is non-poisonous and sticky enough to hold flies alighting on it may be made using the following:

Formula

Coconut oil	50 cc
Refined almaciga	40 gm
Rosin	10 gm
Glycerin	30 cc
Honey	25 gm

Preparation.—Heat the coconut oil to a boil in an aluminum or enamelled pot. Add the almaciga and rosin gradually, stirring the mixture until all the resin has been melted or dissolved. Stir above slowly to avoid excessive foaming. Remove from fire, incorporating the glycerin and honey and stirring continuously in order to get a homogeneous mixture. Coat the Kraft paper sheets cut to a suitable size and preferably brushed with soap water with the mixture while still hot. The coating may be smoothed out with a knife or piece of flat wood. Set aside to cool. If a heavier coating is desired, increase the amount of resin. The paper sheets, once coated, should be laid flat and avoid undue leaf.

"GLASSINE" PAPER

This product is so named because it resembles glass in that it is transparent. It is similar to cellophane although it is slightly colored and somewhat thicker. It retains that usual resin odor and is comparable to waxed paper in texture. It is useful as soap wrappers and finds much demand in drugstores in prescriptions calling for papers.

Formula

Refined almaciga	100 gm
Alcohol	350 cc
Camphor	10 gm
Castor oil	20 cc

Preparation.—With the aid of heat, dissolve the almaciga in alcohol. Allow mixture to cool. Incorporate the camphor dissolved in alcohol and add the castor oil to the solution. Immerse the papers into the solution and allow to dry by hanging the strips in air.

COCONUT CHARCOAL BRIQUETTES

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As verified by studies made by the authors, coconut shell charcoal has a heating value of 7,500 to 7,600 calories per gram dry basis. Although it has a high heating value, however, coconut shell charcoal is somewhat brittle and easily pulverized, making it difficult to store, transport and handlo. These characteristics discourage its wide use for domestic purposes, such as for cooking and ironing. Handling and transporting difficulties probably explains why it is relatively expensive in the market.

Loose coconut shell charcoal with its inherent disadvantages can be improved greatly by briquetting. This process consists of grinding the material and molding it into blocks with binding agents. Asphalt pitch and pitch from coal tar or water gas tar have all been used as binders. Breitmayer and West have used wood tar for this purpose in their charcoal briquetting studies. These materials are not easily available in the Philippines, but starch and other related materials are available and should prove useful as binding agents.

This investigation was undertaken to determine the various factors that affect briquette strength with the use of corn starch as a binding agent. Of the various factors involved, the amount of starch binder, the particle size and the amount of water used in mixing were given particular attention. Starch was used in this work because preliminary observation have shown that a reasonably coherent block could be obtained even with one per cent starch. Another reason is that starch burns without giving off any undesirable odor, an important factor in home cooking.

PROCEDURE

The experimental briquettes were made by grinding coconut shell charcoal, sieving and adding the ground material to a water-starch mixture, and molding and drying the sane. The water-starch mixture was prepared by treating a definite weight of starch with hot water so as to form a mucillagenous mass. In the molding operations, hand pressure was applied to form the briquettes. The briquettes were then dried at 105°C until the

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moisture content of the finished products ranged from 4 per cent to 6 per cent.

Table 1 shows the effect of various amounts of starch binder on the crushing strength of charcoal briquettes. In these

TABLE 1.—*Increase of crushing strength of charcoal briquettes with the increase in the percentage of the starch binder.*

Starch binder	Average crushing strength
Per cent	*lbs. per sq. in.*
1.0	20
2.5	60
5.0	140
7.5	180

tests, the percentages of starch were based on the combined weight of starch and charcoal used. The water added in all the trials made was kept constant at 50 per cent by weight of the charcoal alone, and the particle sizes used in all cases were those passing through a 20-mesh sieve but retained by a 40-mesh sieve (Tyler standard screen). It was observed that the briquettes made with as low as one per cent starch were sufficiently coherent.

The effect of particle size on crushing strength was also investigated. Briquettes composed of uniform size particles, as well as briquettes composed of various sizes of particles were prepared and tested. The results of these tests are shown in Table 2. In this table, the particle sizes of the briquettes marked "conglomerate" consisted of the following by weight:

TABLE 2.—*Effect of particle size on the crushing strength of charcoal briquettes.*

Particle size	Crushing strength
Tyler screen mesh	*lbs. per sq. in.*
P. 10 N.P. 20	75
P. 20 N.P. 40	60
P. 40 and Finer	65
Conglomerate	80

	Per cent
Passing 4-mesh but retained by 10-mesh	15.5
Passing 10-mesh but retained by 20-mesh	34.0
Passing 20-mesh but retained by 40-mesh	18.5
Passing 40-mesh and finer	32.0

RESULTS AND DISCUSSION

The water used and the starch content in all the briquettes tested in Table 2 were kept constant at 50 per cent and 2.5 per cent respectively. From the results of the tests shown in this table, it appears that with the low pressures applied, particle size does not influence significantly the crushing strength of the finished briquettes.

On the other hand, the amount of water used in making the water-starch mixture seems to have a decided effect on the strength of the dried briquettes. It may be observed from Table 3 that as the amount of water used in mixing was increased from 41 to 68 per cent, there was a corresponding increase in crushing strength from 55 pounds to 95 pounds per square inch. The particle size constituting these briquettes were kept the same (passing through 20-mesh Tyler Standard Screen). The starch content was maintained constant at 2.5 per cent of the weight of the charcoal used.

An even more pronounced effect was observed with 62 per cent water in Table 3. The size of the particles composing

TABLE 3.—*Effect of amount of water on the crushing strength and bulk values of charcoal briquettes.*

Water [1]	Crushing strength	Bulk value
Per cent	*lbs. per sq. in.*	*cu. in. per lb.*
41	55	33
58	80	30
68	95	32
62	140	31

[1] Percentage added based on the weight of charcoal.

this briquette was the same as those on conglomerate particles in Table 2. It may be observed that with 50 per cent water, the crushing strength of the briquette was 80 lbs. per square inch (Table 2) but with 62 per cent water, the crushing strength increased to 140 lbs. per square inch with all other factors kept the same in all cases. This only serves to show that water used in mixing, prior to drying, is an important factor in briquetting the charcoal.

Measurements show that loose coconut shell charcoal varying in size from one inch to two and a half inches has a bulk value of 90 cubic inches per pound. Charcoal ground to 10-mesh (Tyler standard screen) has a bulk value of 44 cubic inches per pound. As shown in Table 3, briquetted charcoal,

however, has a bulk value one-third that of loose coconut shell charcoal. This is another important advantage of briquetted charcoal.

SUMMARY

1. Briquetted coconut shell charcoal has many advantages over loose coconut shell charcoal. It is clean and easily handled with its greatly reduced bulk.

2. As low as one per cent starch is sufficient to form a coherent block and the crushing strength of the briquette increases with a corresponding increase in the percentage of starch.

3. It seems that, within the range studied, particle size does not affect significantly crushing strength.

4. The amount of water used in mixing influences the crushing strength of the briquettes.

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HYPHAL PENETRATION OF THE STAINING FUNGUS CERATOSTOMELLA SP. IN RATTAN AND ITS SIGNIFICANCE IN CONTROLLING STAIN

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ONE PLATE AND TWO TEXT FIGURES

A recent survey by Mendoza (1956) indicates that rattan collectors in the different rattan cutting areas and processing plants in Mindanao sustain losses from stain damage ranging from 30 to 60 per cent of the commercial value of the cut poles. This huge loss shows that stain is an important problem in the rattan industry, and its control deserves a little more attention.

Several attempts have been made to find a solution to the rattan stain problem. While some of them were effective in reducing stain, almost all were preliminary and exploratory in nature.

Stain in rattan is caused by fungi, the majority of which belong to *Ceratostomella,* and which are similar to those causing stain in wood. In wood, the staining fungi grow and develop vigorously in newly sawn or green lumber. Similarly, in rattan they grow very rapidly after starting. According to Roldan (1956), fungal entrance into the pole is commonly through its cut ends. Since its internal tissues are very highly porous, once the pole is infected through these ends, hyphal penetration by the staining fungus is very rapid especially through the vessels. In sapwood of shortleaf pine (*Pinus echinata*), Lindgren (1942) found that the approximate daily hyphal penetration in the longitudinal direction by *C. pilifera* was 4.3 mm. He concluded that a delay of more than one day in the application of preventive treatment in practice is not advisable if control of *C. pilifera* is to be expected under all conditions.

The present study of rattan stain concerns the rate of growth in the longitudinal direction in rattan pieces of the staining fungus *Ceratostomella* sp. and its significance in the control of the stain.

HYPHAL PENETRATION STUDY

Methods.—Freshly cut rattan poles having 2 to 3 cm diameters and free from any apparent defects were selected and cut into pieces 30 cm long. The moisture content was taken soon after cutting. One end of each test piece was dipped in hot paraffin liquified at 80 to 90°C, cooled and immediately redipped at a temperature of 50 to 60°C to insure a complete coating. The unparaffined end was disinfected with an aqueous

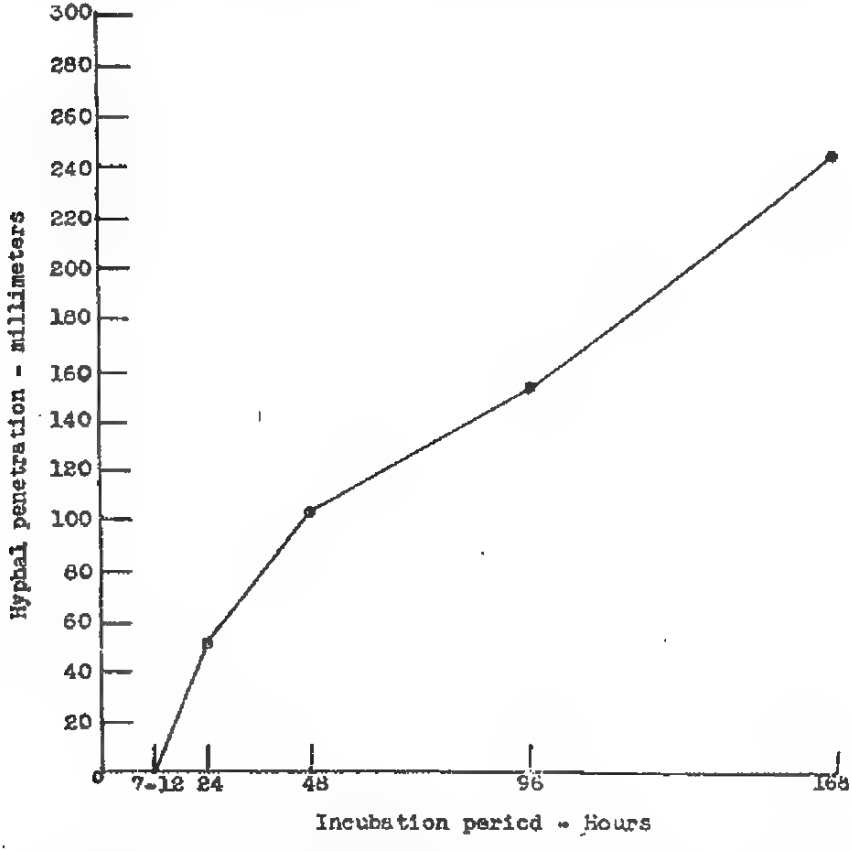

Fig. 1. Trend of hyphal penetration during the different periods of incubation.

mercuric chloride solution diluted to 1:1000 and the end rinsed thoroughly with sterile water. With the aid of the loop of an inoculating needle, the exposed ends of the test pieces were smeared with a spore suspension in sterile water. The inoculated samples were then placed on end (coated end down) in sterile beakers and incubated in a sterilized desiccator with the lid kept partially open to avoid undue accumulation of

moisture. The temperature and humidity of the desiccator chamber were recorded.

After each incubation period of 7 to 12 hours, 48 hours, 96 hours, and 168 hours, a set of samples was removed from the desiccator and split open. Cubes 4 mm in size were cut from the split samples in a series starting from the infected end, and free-hand sections of 100 to 150 microns thickness were prepared and examined under the microscope for the presence of fungus hyphæ. No special staining was needed as the presence of the mycelium was clearly visible under the microscope (Plate 1, fig. 3). The rate of penetration was computed from the number of cubes in which the mycelium was found present.

Results.—The results of the experiments are summarized in Table 1. It will be noted that after the incubation period of

TABLE 1.—*Rate of hyphal penetration of Ceratostomella sp. in rattan.*

Incubation period	Atmospheric condition of incubation chamber		Average moisture content of sample[2]	Penetration[1]			Remarks
	Temperature	Relative humidity		Max.	Min.	Ave.	
Hours	°C	*Per cent*	*Per cent*	*mm*	*mm*	*mm*	
7-12	28.0-30	70-80	139.3	0	0	-------	Hyphal penetration not yet visible.
24	25.5-30	70-84	123.3	55	47	51.0	Hyphal clearly visible in the parenchyma and in the fiber cells.
48	29.0-30	70-84	116.8	108	98	103.0	Hyphae conidiophores and spores abundant in the vessels.
96	28.0-30.5	66-78	108.4	158	147	152.5	
168	29.0-30.5	68-80	102.0	255	235	245.0	

[1] Average of five test samples.

[2] Based on percentage of oven-dry weight.

7 to 12 hours, hyphal penetration was nil. However, after the 24-hour incubation period, penetration had extended to a distance of 51 mm and progressed rapidly thereafter, although the rate of advance tended to diminish somewhat as the incubation period was prolonged. At the end of the 168-hour or 7-day incubation period, the total distance of penetration was 245 mm. The trend of hyphal penetration during incubation period, the approximate average daily rate of hyphal penetration was 32 mm.

CHEMICAL PREVENTIVE TREATMENT OF STAIN

Methods.—The test samples were selected and prepared in the same manner as described in the penetration study. The cut ends were disinfected and smeared with a spore suspension

of a young culture of the staining fungus. The moisture content was taken. The test samples were then incubated in a large desiccator in the same manner as in the previous experiment. Temperature and relative humidity were recorded. At the end of each incubation period of 7 to 12 hours, 24 hours, 48 hours, 96 hours, and 168 hours, a set of samples was removed from the desiccator, scraped, weighed, and dipped horizontally in a solution of 0.84 per cent sodium pentachlorophenate (Dowicide G.) for 2 minutes. This is the concentration of the solution recommended by the chemical manufacturer. The samples were aerated after the treatment and dried for a few hours, then incubated for 7 weeks in a temperature-controlled chamber at 30°C and 80 per cent relative humidity. At the end of 7 weeks, sample were removed from the chamber and the incidence and intensity of stain and per cent moisture content were determined. This experiment was performed in two series.

TABLE 2.—*Chemical preventive treatment of rattan.*[1]

Incubation period	Atmospheric condition of incubation chamber		Average moisture content based on percentage of oven-dry weight of samples after 7 weeks	Infection[1]	
	Temperature	Relative humidity			
Hours	°C	*Per cent*	*Per cent*	*Per cent*	*Intensity*[2]
SERIES I:					
7–12	28.5	78–80	38.2	—	—
24	28.0	80	36.4	100	++
48	28.0	76–80	40.8	100	+++
96	28.5	78–80	37.4	100	++++
168	28.0	72.80	35.3	100	++++
SERIES II:					
7–12	29.0	76–80	37.5	—	—
24	28.5	74.80	36.6	100	+
48	28.0	70–80	40.1	100	++
96	28.0	76–80	35.4	100	+++
168	28.5	70–80	38.8	100	+++

[1] Average of five test samples in each series
[2] Marks mean: — None
+ Light
++ Moderately heavy
+++ Heavy
++++ Very heavy

Results.—The results of the experiment are summarized in Table 2. It will be noted that none of the inoculated samples incubated for 7 to 12 hours, treated, and again incubated for 7 weeks developed stain (Plate 1, fig. 1, a). On the other hand

all samples treated after incubation periods of 24 hours or more, developed stain infection in spite of the chemical treatment (Plate 1, fig. 1). The results of the two studies clearly indicate that while freshly cut poles are susceptible to infection, no stain is likely to develop if treatment is applied within 12 hours after cutting, and that treatment after 24 hours or more after cutting is not effective.

CHEMICAL PENETRATION TEST

Methods.—Samples from freshly cut poles were selected and prepared as in the preceding tests and piled in the laboratory. After periods of 7 to 12 hours, 24 hours, 48 hours, 96 hours, and 168 hours, samples from each set were removed from the pile and scraped. After taking their moisture content, the samples were dipped horizontally for 2 minutes in a solution of 0.84 per cent sodium pentachlorophenate deeply colored with green ink. After dipping, each samples was split open and the depth of penetration of the chemical from the end of the test sample was measured. Penetration was readily determined with the aid of the green color of the solution which, it was assumed, penetrated as far as the chemical. Similar tests were also conducted in which the samples were dipped vertically instead of horizontally. Penetration from the surface through the rind was not observed to have taken place in these tests.

TABLE 3.—*Chemical penetration in rattan.*

Hours after cutting	Horizontal dipping		Vertical dipping	
	Average moisture content of the sample [1]	Penetration [2]	Average moisture content of the sample [1]	Penetration [2]
	Per cent	*mm*	*Per cent*	*mm*
7–12	122	0.0–2	128	2–3
24	129	1.5–2.5	135	2–8
48	123	2.0–3	118	3–12
96	117	2.0–4	107	5–15
168	105	2.0–6	98	5–28

[1] Based on percentages of oven-dry weight.
[2] Average of 5 samples for each test.

Results.—As summarized in Table 3, depths of penetration range from 0 to 2 mm when horizontally-dipped poles were treated 7 to 12 hours after cutting, and 2 to 3 mm in the case of vertical dipping. When treated 24 to 168 hours after cutting, the depth of penetration range was 1.5 to 6 mm with

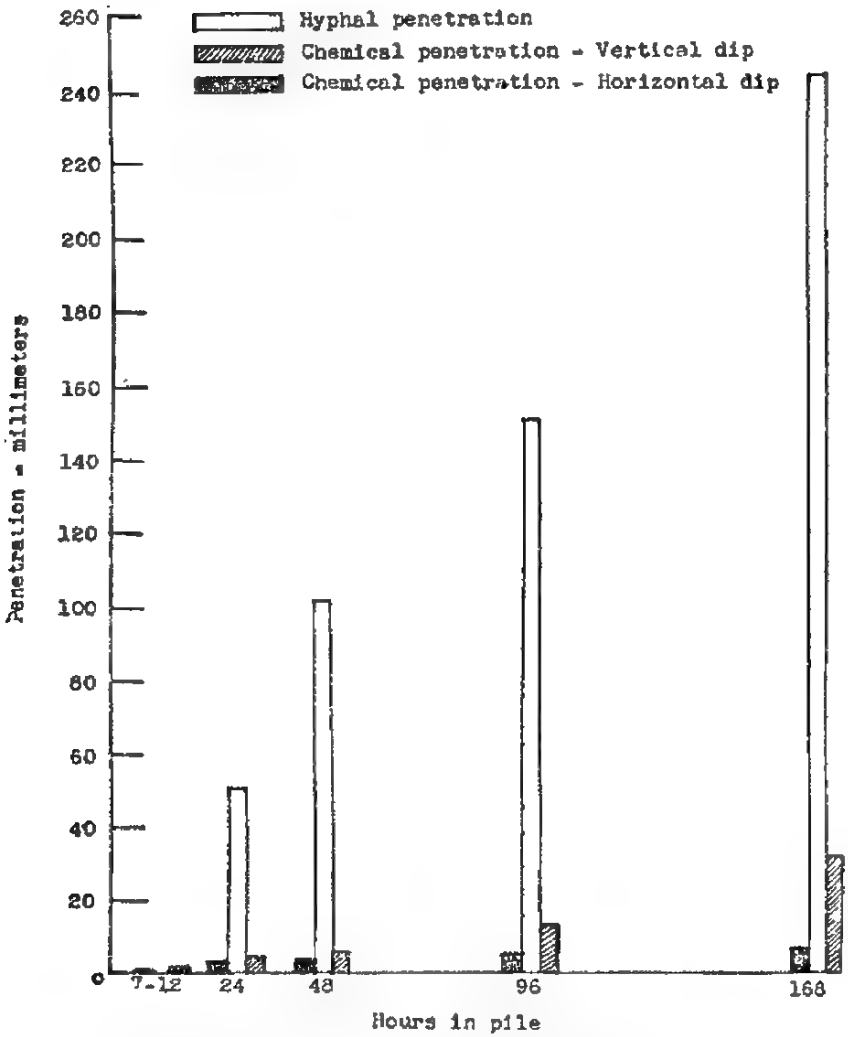

FIG. 2. Comparison of rates of hyphal and chemical penetration in rattan.

horizontal dipping, and 2 to 28 mm with vertical dipping. By comparing chemical penetration with hyphal penetration by the staining fungus, it is obvious that fungal penetration in rattan, after it has been infected for 24 hours, is far ahead of the chemical penetration that would be provided by dipping. Figure 2 was prepared to facilitate comparison of depths of hyphal and chemical penetration in rattan. With this as a basis, practical and effective preventive measures for stain control can be formulated. To be effective in preventing stain, the

chemical treatment of rattan should be accomplished within 12 hours after cutting, unless the poles are completely protected from stain infection while awaiting treatment. Further delay may render the treatment completely ineffective (Plate 1, fig. 2, b and c).

SUMMARY AND CONCLUSION

The staining fungus, *Ceratostomella* sp., readily infects rattan. The fungus enters through the ends of the freshly cut poles. However, penetration is not appreciable during the first 12 hours following infection. Treatment within 12 hours after cutting is therefore necessary, if control of stain is desired. Because the growth of the fungal hyphæ is rapid, the penetration extends as much as 51 mm in the longitudinal direction 24 hours after infection and a further delay in the application of a preventive chemical renders the treatment completely ineffective.

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ILLUSTRATION

PLATE 1

FIG. 1. Comparative stain development on the surface of rattan samples treated at different periods of time after cutting and inoculation: (*a*) 7 to 12 hours, (*b*) 24 hours, (*c*) 48 hours, (*d*) 96 hours, and (*e*) 168 hours.

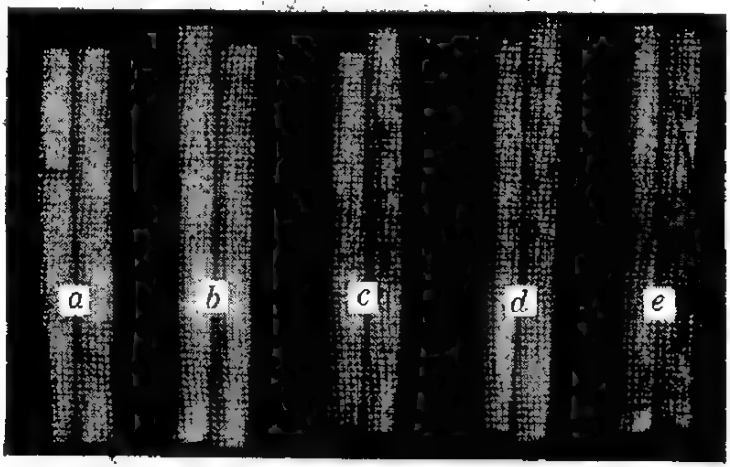
2. Internal development of stain in specimens treated at different periods of time after cutting and inoculation: (*a*) 7 to 12 hours, (*b*) 24 hours, and (*c*) 48 hours.

3. Cross and longitudinal sections of the internal tissue of rattan infected with *Ceratostomella* sp.: (*a*) Hyphæ, conidiophores and spores of the fungus in the vessel, (*b*) Hyphæ of the fungus in the fiber cells.

TEXT FIGURES

FIG. 1. Trend of hyphal penetration during the different periods of incubation.

2. Comparison of the rates of hyphal and chemical penetration in rattan.



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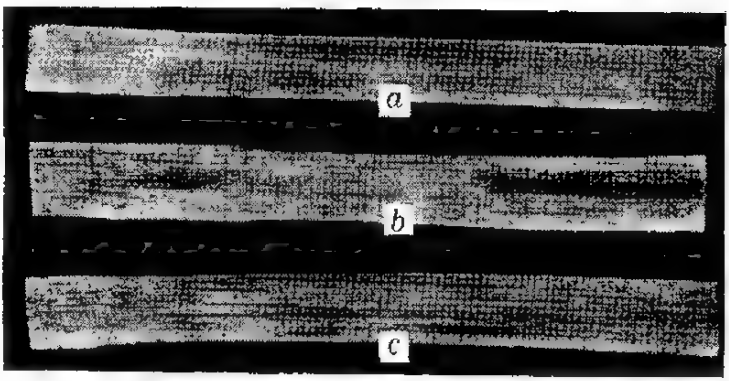

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PLATE 1.

NOTES ON PHILIPPINE MOSQUITOES, XX

DAYTIME OBSERVATIONS IN HOUSES OF TWO BARRIOS IN LAGUNA PROVINCE[1]

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There are puzzling variations in mosquito behavior: certain aspects in the habits of a species often differ in different localities. This may lead to contradictory opinions, specially when workers deal with the same species in separate, though neighboring, areas. Probably there are imperceptible variations in environmental factors to which the mosquitoes respond in easily detectable ways.

Local workers, just like workers elsewhere, have had uncomforting experiences; uncomforting in the sense that they did lead to misunderstanding—sometimes. To those whose activities were confined in the relatively large Island of Luzon, it was an established fact, based on numerous data gathered through the years in many parts of this island, that *minimus flavirostris* was a "wild" species, since it was very seldom found resting in houses during the day. Unfortunately or fortunately (may be), those who studied the behavior of this mosquito in Mindoro and Mindanao islands obtained what they believed to be equally incontrovertible evidences that *flavirostris* was a "domestic" species, because it could be caught quite readily in houses any time of day. Well, later on, as observations progressed, it became clear that neither the one nor the other of the conflicting opinions was exactly correct. For there were places (very exceptional, indeed, and few in Luzon; quite many in Mindanao) where the previously observed facts did not hold good. Right in the heart of Laguna Province (Luzon Island), in rural barrios of San Pablo City (to be exact), *flavirostris*

[1] Help from many people, either directly or indirectly obtained, made these observations possible. On top of all, of course, was our Director, Antonio Ejercito, M.D., down through the line to field men as well as laboratory technicians and typists. To mention only a few: Mr. Oscar Baldovino, Mr. Graciano Balcita and Mr. Provenir Jaro, field technicians; Miss Marina Arjona, who assisted in the identification of catches; Mr. Jose Santos, Mr. Felix V. Dantis, and Mr. Daniel T. Alejandria, laboratory personnel at Tala.

could be caught in a few houses any day. On the other hand, in the large Province of Bukidnon (not to mention other places) in Mindanao Island, hardly any *flavirostris* could be found in houses at the daytime. Yet when the population of *flavirostris* was measured by means of carabao-baited night traps, the density was higher in places where very few or none was found in houses (Bukidnon Province) than those where *flavirostris* often rested indoors (Cotabato Province). This did not apparently indicate the absence of indoor-frequenting *flavirostris* in Bukidnon; probably, it merely indicated unknown factors which influenced the behavior of *flavirostris* in houses in this respect.

While thus trying to gain a better understanding of the habits of vector species, something about the indoor behavior of other mosquitoes was also incidentally learned. This was specially the case in places like Manila, where Institute of Malariology personnel (including the senior author) were required to participate actively in mosquito pest control. For instance, it would be nearly 100 per cent correct to say that in Manila *Culex fatigans*[2] alone habitually stays in houses during the day. Now and then, at long intervals or at certain seasons and in particular sections of the city, *Aedes aegypti, Aedes albopictus, Culex tritaeniorrhynchus summorosus,*[3] *Anopheles limosus,* and *Anopheles litoralis* rest indoor during the day. Still much more rarely. *Armigeres baisasi, Culex gelidus, Culex incognitus, Culex whitmorei, Toxorhynchites splendens, Mansonia annulifera, Mansonia uniformis,* and *Uranotaenia atra* (primarily a sylvan species) somehow found their way into Manila homes. Adults of a pitcher plant-breeding *Tripteroides* (Group A) were once caught in a house located in the heart of Manila, no doubt merely brought with this decorative plant (*Nepenthis*) from its jungle habitat.

[2] The correct scientific name is *Culex quinquefasciatus* Say, 1823. *See* Stone, Alan. Corrections in the taxonomy and nomenclature of mosquitoes. Proc. Ent. Soc. Wash. 58 (1956) 342. *Fatigans* is used here merely for convenience.

[3] For an opinion regarding *Culex summorosus, see* Baisas, F. E. Notes on Philippine mosquitoes, VII. *Culex* (*Culex*) with banded proboscis and tarsi. Monthly Bull. Bureau of Health 18 (1938) 196–200. *Also* Colless, D. H. Notes on culicine mosquitoes of Singapore, II. The *Culex vishnui* group (Diptera, Culicidæ) with descriptions of two new species. Ann. Trop. Med. Parasit. 51 (1957) 98–100.

Actually, however, the picture differs markedly in rural areas, though sometimes surprisingly contradictory to obvious factors. The low-lying City of Manila, largely reclaimed from swamps as it is, cannot get rid of *fatigans* simply because it cannot get rid of its numerous dirty canals. And yet the beautiful, clean City of Baguio may be termed "Fatigans' City," too. In its streams and canals, specially those polluted by house refuse or washings, and in leakages of its sewage pipes, *fatigans* breeds as heavily as in the Manila esteros.

Along salt water sea coasts, where conditions of city slums are absent and where pollution of fresh water is less, *Culex fatigans* may be so few as to escape notice altogether. There are exceptions, of course: in certain communities at the very edge of the sea, such as the highly filarious town of Santa Magdalena, Sorsogon Province, *C. fatigans* is almost the only species found in houses at all seasons. The total, however, is far smaller than the usual large number frequenting Manila homes.

Where fresh-water lakes, ponds, marshes, slews and the like are present, the vicious-biting *Mansonia uniformis* may camouflage the presence of *fatigans* and other house-frequenting species at least at certain times of the year. Or one may be unduly alarmed by the large number of different mosquitoes, including anophelines, that suddenly invades homes in the evening when there is a gathering storm; or by the hundreds of *Culex whitmorei* that rest on walls of houses in particular evening (where this species is very numerous at certain months: September to November), but without any apparent desire to feed on the occupants.

Wire screens, when used to protect house owners in rural areas, provide means of observing particular aspects of mosquito behavior. At the onset of the dry season, for instance, way back in 1924 to 1926, hundreds of *indefinitus* and *limosus*, some *philippinensis*, a few *annularis* and *ludlowæ* were often noted early in the morning resting on the outside of screens of doors and windows of the Pampanga Sugar Mills Hospital at Del Carmen. This was a concrete building. At the onset of the rainy season a considerable number of *tessellatus* was likewise observed in the same situation. Inside the houses nearby and in barrios, not many mosquitoes except sometimes *tessellatus*, were observed at night or during the daytime.

Screened windows and doors in living quarters at Tala (Institute of Malariology) show something quite different. Almost all the year round, *fatigans* may be seen attempting to enter, from dusk and throughout the night to early morning. Some successfully squeeze themselves through the wire mess (16 by 16 per linear inch) or else find openings in the frame-work and wood fittings sufficiently wide for ingress, and these few could be so maddening when one is not in too good humor. *Flavirostris, filipinæ, limosus, peditaeniatus* and *barbirostris-manalangi* are, in certain nights, observed outside the wire screens when a room is brightly lighted by flourescent lamps. (These species were determined by sample catches). Curiously, too, in February and March or earlier, a number of fully-blooded *Aedomyia catasticta* rests on the outside of such screens from early morning till late in the morning or afternoon stirring only when direct sunlight hits them. This species has not been caught in houses during the day in catching areas scattered in different parts of the Islands. There was a time, however, when, together with other Division of Malaria personnel, the senior author was invited to visit the Pilot DDT Project in Mindoro Island; quite a few *catasticta* were caught during the day in what Dr. Sambasiban and Dr. Bathia then called *flavirostris*-houses (i.e. houses highly frequently by *flavirostris*) in their observation areas. Lately, in the course of collecting "H-fever mosquitoes" in Hagonoy, Bulacan, more than a dozen *catasticta* were caught indoors during the day.

Obviously, years of observations by many workers in various parts of the Philippines will be the only way to gather sufficient and accurate information concerning the bionomics of Philippine mosquitoes.

As a preliminary to whatever may be done later along this line, two villages in Laguna Province were chosen for a year's observation (March, 1955 to February, 1956) of mosquitoes in houses during the day. They were particularly selected for contrast. Barrio San Antonio of San Pablo is situated in a region planted to coconuts miles and miles around. A considerable number of lansones, some cacao plants, coffee and bananas thrive in between the rows of coconuts. The place, moreover, is about 350 feet above sea level. But the most important reason was that certain houses in this barrio were highly frequented by *flavirostris* during the day. Barrio Masiit, of the Municipality of Calauan, on the other hand, is hardly half a dozen feet above sea level. It is completely surrounded by

open rice fields, although in the yards of the closely grouped houses, bananas, a few lansones, and some coconuts grow luxuriantly. Owing to excellent irrigation, two crops of rice a year are produced in the paddies there. Houses in Barrio San Antonio had not been subjected to DDT-spraying, excepting after these observations were completed; whereas the houses in Barrio Masiit had already received, by the time the observations were about to terminate, three consecutive yearly DDT-treatments. Twenty-five kilometers of good road (probably only 15 kilometers by straight line, a part of the road being full of zig-zags) separates the two barrios.

Barrio Masiit was one of the very first villages chosen for malaria and mosquito studies by the International Health Board of the Rockefeller Foundation way back in 1924. It was, however, abandoned after a few months for other similarly malarious communities in Pampanga Province. It was again chosen as site for study by other Rockefeller Foundation men (1930–34), the famous malariologist, and now special consultant to the World Health Organization Expert Committee on Malaria, Paul F. Russell, and the top entomologist, W. V. King. After World War II, the United States Public Health Services (Malaria Control Division in the Philippines) subjected the houses in Barrio Masiit and the houses of the town proper to DDT-spraying (5 per cent in kerosene).

Barrio Masiit and its mother town were remarkable for the fewness, almost complete absence, of *flavirostris* in houses during the day. This was true at the time of Dr. Russell and Dr. King, and thereafter. In later years, workers in various other parts of the Philippines found *flavirostris* quite frequently indoors at the daytime. These new workers, therefore, thought that the results of previous observations (by Russell, King, Manalang, Ejercito, Urbino, etc.) were misleading. However, even at present in parts of Laguna Province not sprayed with DDT, *flavirostris* is very seldom found indoors during the daytime. (The only known exceptions are the few houses in San Pablo City cited above.) This is equally true in many, many other localities of Luzon Island, as well as in many places in the Bisayan provinces.

No previous malaria or mosquito observations were done in Barrio San Antonio. Houses in this village were more in number than, and superior in construction to, those in Barrio

Masiit. But the point of investigation was to find what mosquitoes might be harboring in houses during the day—so the differences in number of homes was considered unimportant. Ingress and egress of mosquitoes in well-built houses seem to differ little, if at all, from movements of these insects in grass or palm shacks.

The mosquito fauna of these two villages, based on larvæ collected from all discovered breeding waters, and from those reported by previous workers, differed only in number of individuals and not in species. Sylvan and semi-sylvan species, axil breeders, breeders in ground waters, and so on are present in both. Differences in adults caught indoors may thereby be considered merely due to artificial factors: DDT residue in houses of Masiit, for instance; differences in major vegetation: rice in Masiit and coconuts in San Antonio. The following species were credited to these barrios:

1. *Aedes* (*Aedes*) *dux* Dyar and Shannon, 1925
2. *Aedes* (*Aedes*) *margarsen* Dyar and Shannon, 1925
3. *Aedes* (*Aedes*) *nigrotarsis* (Ludlow), 1908
4. *Aedes* (*Aedimorphus*) *alboscutellatus* (Theobald), 1907
5. *Aedes* (*Aedimorphus*) *pampangensis* (Ludlow), 1905
6. *Aedes* (*Aedimorphus*) *vexans nocturnus* (Theobald), 1903
7. *Aedes* (*Banksinella*) *lineatopennis* (Ludlow), 1905
8. *Aedes* (*Finlaya*) *abadsantosi* Baisas, 1946
9. *Aedes* (*Finlaya*) *flavipennis* (Giles), 1904
10. *Aedes* (*Finlaya*) *luteus* (Ludlow), 1905
11. *Aedes* (*Finlaya*) *niveus* (Ludlow), 1903
12. *Aedes* (*Finlaya*) *poicilius* (Theobald), 1903
13. *Aedes* (*Muscidus*) *ferinus* Knight, 1947
14. *Aedes* (*Stegomyia*) *aegypti* (Linnæus), 1762
15. *Aedes* (*Stegomyia*) *albopictus* (Skuse), 1894
16. *Aedes* (*Stegomyia*) *arboricolus* Knight and Rozeboom, 1946
17. *Aedes* (*Stegomyia*) *desmotes* (Giles), 1904
18. *Aedes* (*Stegomyia*) *gardneri* (Ludlow), 1905
19. *Aedes* (*Stegomyia*) *meronephada* (Dyar and Shannon), 1925
20. *Aedes* (*Stegomyia*) *scutellaris* (Walker), 1858
21. *Anopheles* (*Anopheles*) *aitkeni bengalensis* Puri, 1930
22. *Anopheles* (*Anopheles*) *insulaeflorum* (Swell. and Swell.), 1919
23. *Anopheles* (*Anopheles*) *barbirostris* van der Wulp, 1884[4]

[4] Dr. J. P. Reid, senior entomologist, Institute for Medical Research, Kuala Lumpur, Malaya, is now engaged in the study of the entire *barbirostris* complex. Preliminary information from him regarding Philippine materials lent to him for his study indicates that the Philippine forms may be entirely different from the *barbirostris* of van der Wulp.

24. *Anopheles* (*Anopheles*) *hyrcanus lesteri* Baisas and Hu, 1936
25. *Anopheles* (*Anopheles*) *hyrcanus peditaeniatus* Leicester, 1908
26. *Anopheles* (*Anopheles*) *hyrcanus pseudosinensis* Baisas, 1935
27. *Anopheles* (*Anopheles*) *pseudobarbirostris* Ludlow, 1902
28. *Anopheles* (*Anopheles*) *manalangi* Mendoza, 1940 *
29. *Anopheles* (*Myzomyia*) *annularis* van der Wulp, 1884
30. *Anopheles* (*Myzomyia*) *filipinae* Manalang, 1930
31. *Anopheles* (*Myzomyia*) *flavirostris* (Ludlow), 1914
32. *Anopheles* (*Myzomyia*) *karwari* (James), 1903
33. *Anopheles* (*Myzomyia*) *kochi* Donitz, 1901
34. *Anopheles* (*Myzomyia*) *ludlowæ* (Theobald), 1903
35. *Anopheles* (*Myzomyia*) *maculatus* Theobald, 1901
36. *Anopheles* (*Myzomyia*) *mangyanus* (Banks), 1906
37. *Anopheles* (*Myzomyia*) *philippinensis* Ludlow, 1902
38. *Anopheles* (*Myzomyia*) *subpictus indefinitus* (Ludlow), 1904
39. *Anopheles* (*Myzomyia*) *tessellatus* Theobald, 1901
40. *Anopheles* (*Myzomyia*) *vagus limosus* King, 1932
41. *Aedomyia catasticta* Knab, 1909
42. *Armigeres* (*Armigeres*) *malayi* Theobald, 1901
43. *Armigeres* (*Armigeres*) *manalangi* Baisas, 1935
44. *Armigeres* (*Armigeres*) *baisasi* Stone and Thurman, 1958
45. *Armigeres* (*Leicesteria*) *flavus* (Leicester), 1908
46. *Armigeres* (*Leicesteria*) *magnus* Theobald, 1908
47. *Culex* (*Culex*) *annulirostris* Skuse, 1889
48. *Culex* (*Culex*) *annulus* Theobald, 1901
49. *Culex* (*Culex*) *bitaeniorhynchus* (complex) Giles, 1901
50. *Culex* (*Culex*) *quinquefasciatus* Say, 1823
51. *Culex* (*Culex*) *fuscocephalus* Theobald, 1907
52. *Culex* (*Culex*) *gelidus* Theobald, 1901
53. *Culex* (*Culex*) *incognitus* Baisas, 1938
54. *Culex* (*Culex*) mimulus Edwards, 1915
55. *Culex* (*Culex*) *nigropunctatus* Edwards, 1926
56. *Culex* (*Culex*) *sinensis* Leicester, 1908
57. *Culex* (*Culex*) *summorosus* Dyar, 1920
58. *Culex* (*Culex*) *whitmorei* Giles, 1904
59. *Culex* (*Lophoceraomyia*) *fraudatrix* (Theobald), 1905
60. *Culex* (*Lophoceraomyia*) *infantulus* Edwards, 1922
61. *Culex* (*Lophoceraomyia*) *mammilifer* (Leicester), 1908
62. *Culex* (*Lophoceraomyia*) *pachecoi* Baisas, 1935
63. *Culex* (*Lophoceraomyia*) *rubithoracis* Leicester, 1908
64. *Culex* (*Lutzia*) *fuscanus* Wiedemann, 1820
65. *Culex* (*Lutzia*) *halifaxi* Theobald, 1903
66. *Culex* (*Mochthogenes*) *malayi* (Leicester), 1908
67. *Culex* (*Neoculex*) *brevipalpis* (Giles), 1902
68. *Ficalbia* (*Etorleptiomyia*) *elegans* Taylor, 1929
69. *Ficalbia* (*Etorleptiomyia*) *luzonensis* Ludlow, 1905
70. *Ficalbia* (*Mimomyia*) *chamberlaini metallica* (Leicester), 1908
71. *Ficalbia* (*Ravenolites*) *deguzmanæ* Mattingly, 1956

* See footnote on page 52.

72. *Hodgesia malayi* Leicester, 1908
73. *Hodgesia quasisanguinae* Leicester, 1908
74. *Malaya genurostris* (Leicester), 1908
75. *Mansonia* (*Mansonioides*) *annuliferus* Theobald, 1901
76. *Mansonia* (*Mansonioides*) *uniformis* Theobald, 1901
77. *Orthopodomyia* (*Anopheloides*) *anopheloides* (Giles), 1903
78. *Orthopodomyia* (*Anopheloides*) *madrensis* Baisas, 1946
79. *Topomyia pseudobarbus* Baisas, 1946
80. *Toxorhynchites splendens* Wiedemann, 1819
81. *Tripteroides* (*Tripteroides*) *dyari* Bohart and Farner, 1944
82. *Tripteroides* (*Tripteroides*) *monetifera* Dyar, 1920
83. *Tripteroides* (*Tripteroides*) *nitidoventer* Giles, 1904
84. *Uranotaenia annandalei* Barruad, 1926
85. *Uranotaenia arguellesi* Baisas, 1935
86. *Uranotaenia argyrotarsis* Leicester, 1908
87. *Uranotaenia atra* Theobald, 1905
88. *Uranotaenia lagunensis* Baisas, 1935
89. *Uranotaenia mendiolai* Baisas, 1935
90. *Uranotaenia tubanguii* Baisas, 1935
91. *Uranotaenia simplex* sp. nov.

The houses were visited in the morning. At first it was generally thought the early morning hours were the most suitable for indoor mosquito catching. Subsequent and more careful observations, however, not only in Laguna Province but in many other parts of the Philippines, have shown that mosquitoes found resting indoors early in the morning remain indoors all day unless forcefully driven by human agency or unusual disturbances. There is, thereby, no real advantage in catching very early in the morning when people are still sleeping or else not in the mood to be genial to mosquito-men.

Indoor catching, no doubt, will be best between 9:00 p. m. to 4:00 a.m., but again this cannot be done as often as desired owing to the annoyance it will cause the house occupants.

Twelve minutes were spent searching for, and catching, mosquitoes in each house. In most cases this was rather too long specially when houses were sprayed with insecticide, but followed nonetheless as a test of how practical this "standard time" (introduced by the WHO malaria experts who worked in the Mindoro DDT Pilot Project) might be. The scheduled visits were once every fortnight for each house. However, certain uncontrollable factors made it impossible to visit all houses an equal number of times; rather, there were unfortunate inequalities, resulting from: (*1*) transfer of some houses to places outside the observation areas; (*2*) construction of new ones; (*3*)

suspension of scheduled visits due to clashes between the Army and the dissidents; (*4*) evacuation of some inhabitants to less troubled communities; and (*5*) frequent absence of house owners who were mostly busy farmers who went out to the field early in the morning and came late in the evening.

In all, 94 houses were visited in Barrio San Antonio; 66 in Barrio Masiit; that is, including all that were visited only once to those that were visited 20 or more times. In spite of the larger number of houses in Barrio San Antonio, the total number of visits and the total time consumed in these visits were less than those in Barrio Masiit. That was primarily due to the more unsettled conditions in San Antonio at the time.

TABLE 1.—*Number of houses visited; number of visits made; and time spent in visits.*

Barrio San Antonio, San Pablo City				Barrio Masiit, Calauan			
Number of visits	Number of houses	Total visits	Minutes spent	Number of visits	Number of houses	Total visits	Minutes spent
1	17	17	204	1	4	4	48
2	13	26	312	2	1	2	24
3	2	6	72	3	2	6	72
4	2	8	96	4	1	4	48
5	4	20	240	5	1	5	60
6				6	1	6	72
7	3	21	252	7	2	14	168
8				8	2	16	192
9	2	18	216	9			
10				10	1	10	120
11	3	33	396	11			
12	2	24	288	12			
13	2	26	312	13	1	13	156
14	1	14	168	14			
15	1	15	180	15	1	15	180
16	2	32	384	16			
17	1	17	204	17	4	68	816
18	6	108	1,296	18	2	36	432
19	5	95	1,140	19	7	133	1,596
20	5	120	1,440	20	1	20	240
21	6	126	1,512	21	6	126	1,512
22	8	176	2,112	22	5	110	1,320
23	5	115	1,380	23	12	276	3,312
24	3	72	864	24	10	240	2,880
25				25	2	50	600
Total	94	1,089	13,068 or 217.8 hrs.	Total	66	1,154	13,848 or 230.8 hrs.

1527 anophelines and 860 non-anophelines were caught in Barrio San Antonio during the period of observation. 48 or 3.14 per cent of the anophelines were males; 1479 or 96.86 per cent were females. 210 or 14.19 per cent of the females were *minimus flavirostris,* of which 165 or 78.57 per cent were blooded, 9 or 4.29 per cent were gravid, and 36 or 17.14 per cent had empty

stomachs. Among the non-anophelines, 294 or 34.19 per cent were males and 566 or 65.81 per cent were females. The conditions of the abdomen of the different species (excepting *indefinitus, limosus, manalangi,* and *pseudobarbirostris*) are shown in Table 2.

TABLE 2.—*Daytime indoor catches in Barrio San Antonio, San Pablo City.*

Species	♂♂	♀♀	Total	Females condition of abdomen			No. positive houses
				Blooded	Gravid	Empty	
Aedes aegypti	201	236	437	97	9	130	64
albopictus	1	8	9	5		3	5
flavipennis		4	4	1		3	4
lineatopennis							
nocturnus		7	7	6		1	7
pampangensis							
poicilius	1	2	3	1		1	3
Armigeres baisasi	1	6	7	2		4	6
Culex annulus		17	17	10		7	8
bitaeniorhynchus		1	1			1	1
brevipalpis							
fatigans	46	87	133	59	2	26	46
fraudatrix		1	1			1	1
fuscanus	1	1	2			1	2
fuscocephalus	3	16	19	9		7	9
gelidus	2	6	8	2		4	6
incognitus	15	83	98	45	1	37	39
malayi-laureti		1	1			1	1
rubithoracis		1	1			1	1
summorosus	19	86	105	48	3	35	40
whitmorei		1	1			1	1
Mansonia uniformis		1	1	1			1
Tripteroides dyari	2		2				2
nitidoventer	1	1	2			1	2
Uranotaenia argyrotaxis	1		1				1
atra							
Total	294	566	860	286	15	265	
Anopheles filipinae		5	5	1		4	4
flavirostris	21	210	231	165	9	36	39
mangyanus	7	31	38	14	3	14	20
maculatus		2	2			2	2
annularis		2	2			2	2
philippinensis		5	5			5	4
kochi		10	10	7		3	8
tessellatus	2	21	23	11		10	11
indefinitus	6	539	545	not recorded			62
limosus	10	581	591	not recorded			67
ludlowae		1	1				1
barbirostris		2	2	1		2	2
lesteri							
manalangi	2	55	57	not recorded			15
peditaeniatus		1	1			1	1
pseudobarbirostris		14	14	not recorded			10
Total	48	1,479	1,527	199	12	79	

In Barrio Masiit, only 179 anophelines and 365 non-anophelines were caught. The males, females, condition of the female abdomen of each species, and positive houses are also shown in Table 2.

TABLE 2a.—*Daytime indoor catches in Barrio Masiit, Calauan.*

Species	♂♂	♀♀	Total	Females condition of abdomen			No. positive houses	Resting on—	
				Blooded	Gravid	Empty		sprayed surface	sprayed surface
Aedes aegypti									
albopictus		1	1						
flavipennis		1	1	1			1	1	
lineatopennis		1	1			1	1		1
nocturnus		6	6	1			1		1
pampangensis		2	2	5		1	6		6
poicilius						2	1		2
Armigeres baisasi		3	3						
Culex annulus		2	2	1		2	3		3
bitaeniorhynchus		1	1	1		1	2		2
brevipalpis		2	2	1			1		1
fatigans	85	203	288	2			1		1
fraudatrix				77		126	44	84*	204
fuscanus									
fuscocephalus	1		1						
gelidus	1	3	4				1	1	
incognitus	6	10	16			3	4		4
malayi-laureti				8		2	11	3	13
rubithoracis									
summorosus									
whitmorei	5	29	34	22		7	18	6	29
Mansonia uniformis		1	1	1			1		1
Tripteroides dyari									
nitidoremier									
Uranotaenia argyrotarsis									
alra	2		2						2
Total	100	265	365	120		145		94	271
Anopheles filipinae		1	1	1			1		1
flavirostris	2	1	3	1			3	1	2
mangyanus									
maculatus									
annularis									
philippinensis									
kochi									
tessellatus	6	17	23	6		11	16	6	17
indefinitus	29	66	95	23		43	27	27*	63
limosus	5	37	42	14		23	19	6	36
ludlowae									
barbirostris	1	1	2			1	2	1	1
lesteri	1	4	5	1		3	3	1	4
manalangi		1	1			1	1		1
peditaeniatus		2	2	2			1		2
pseudobarbirostris		5	5	2		3	5		5
Total	44	135		179	50	85		42	137

* The apparent toxic, or lack of tonix, effect on mosquitoes caught while directly resting on surfaces where traces of insecticides were visible will be discussed in another paper.

Although the much smaller number of catches in Barrio Masiit was undoubtedly due largely to DDT, it would be erroneous to attribute the "reduction" exclusively to this artificial factor; more so, if the catches from Barrio San Antonio were taken as a basis of comparison. While the breeding places and the daytime outdoor refuge of *flavirostris* were practically the same in

extent and conditions in these two areas, factors of major influence to other mosquitoes were not balanced. A lot more breeding waters, for instance, of *fatigans* were present in Barrio Masiit than were existent in Barrio San Antonio, just as there were considerably more artificial containers (mostly coconut shells) in Barrio San Antonio, which *Aedes aegypti* and *Aedes*

TABLE 3.—*Total catches and densities per species.*

Species	Barrio San Antonio, San Pablo City				Barrio Masiit, Calauan			
	Total females	Female density*	Total ♂♂ and ♀♀	Density for both sexes*	Total females	Female density†	Total ♂♂ and ♀♀	Density for both sexes†
Aedes aegypti	286	1.082	437	2.006				
albopictus	8	0.036	9	0.041	1	0.004	1	0.004
flaviventris	4	0.018	4	0.018	1	.004	1	0.004
lineatopennis					1	0.004	1	0.004
nocturnus	7	0.032	7	0.032	6	0.026	6	0.026
pampangensis					2	0.008	2	0.008
poicilius	2	0.009	3	0.014				
Armigeres baisasi	6	0.028	7	0.032	3	0.013	3	0.013
Culex annulus	17	.073	17	0.073	2	0.008	2	0.008
bitaeniorhynchus	1	0.004	1	0.004	1	.044	1	0.004
brevipalpis					2	0.008	2	0.008
fatigans	87	0.391	133	0.610	203	0.879	288	1.248
*fraudatrix***	1	0.004	1	0.004				
fuscanus	1	0.004	1	0.004				
fuscocephalus	16	0.073	19	0.087			1	0.004
gelidus	6	0.028	8	0.036	3	0.013	4	0.016
incognitus	83	0.380	98	0.449	10	0.043	16	0.074
malayi-laureli	1	0.004	1	0.004				
rubithoracis	1	0.004	1	0.004				
summorosus	86	0.390	105	0.482	29	0.125	34	0.147
whitmorei	1	0.004	1	0.004				
Mansonia uniformis	1	0.004	1	0.004	1	0.004	1	0.004
Tripteroides dyari			2	0.008				
nitidoventer	1	0.004	2	0.008				
Uranotaenia argyrotarsis			1	0.004				
alra							2	0.008
Total non-anophelines	566	2.599	860	3.949	265	1.148	365	1.581
Anopheles filipinae	5	0.023	5	0.023				
flavirostris	210	0.964	231	1.061	1	0.004	3	0.013
mangyanus	31	0.142	38	0.174				
maculatus	2	0.009	2	0.009				
annularis	2	0.009	2	0.009				
philippinensis	5	0.022	5	0.022				
kochi	10	0.045	10	0.045				
tessellatus	21	0.096	23	0.106	17	0.074	23	0.099
indefinitus	539	2.474	546	2.668	66	0.286	95	0.412
limosus	581	2.667	591	2.714	37	0.160	42	0.182
ludlowae	1	0.004	1	0.004				
*barbirostris****	2	0.009	2	0.009	1	0.004	2	0.008
lesteri					4	0.016	5	0.022
*manalangi****	55	0.252	57	0.262	1	0.004	1	0.004
pedilaeniatus	1	0.004	1	0.004	2	0.008	2	0.008
pseudobarbirostris	14	0.064	14	0.064	5	0.022	6	0.022
Total anophelines	1,479	6.791	1,527	7.011	135	0.585	179	0.775
Total anophelines and non-anophelines	2,045	9.389	2,387	10.959	400	1.733	544	2.357

* Density or average catch per man per hour, based on 217.8 catching hours.

† Density of average catch per man per hour, based on 230.8 catching hours.

** *C. fraudatrix* is being re-studied by Dr. Colless. According to him the Philippine form may not be *fraudatrix*.

*** *A. barbirostris* complex is under study by Dr. Reid, who says: the Philippine forms differ from *barbirostris* of van der Wulp.

TABLE 4.—*Monthly densities of culicine catches.*

Species	1956 Barrio San Antonio								1956			
	March	April	May	June	July	August	September	October	November	December	January	February
Aedes aegypti	2.40	1.63	0.57	0.69	3.70	1.20	1.25	1.21	2.39	1.83	4.73	1.22
albopictus				0.03			0.05	0.08	0.06	0.25	0.05	
flaviventris			0.03					0.04				
lineatopennis												
nocturnus			0.03	0.06		0.05	0.05					
pampangensis												
poicilius					0.5	0.05		0.08				
Armigeres baisasi												
Culex annulus		0.25	0.09	0.17	0.05	0.05	0.06	0.13			0.05	
bitaeniorynchus	0.05							0.06			0.05	
fatigans	0.35	0.06	0.09	0.06	1.30	0.20	0.10	0.25	0.33	1.58	1.99	2.44
fraudatrix					0.05							
fuscanus					0.05							
fuscocephalus	0.05	0.06			0.15	0.10	0.10	0.17	0.06	0.25	0.05	0.10
gelidus	0.05				0.05	0.20	0.05					
incognitus	0.50	0.25	0.12	0.29	0.65	1.05	1.00	0.38				0.20
malayi-laureli				0.03								
rubithoracis						0.05						
summorosus	0.15		0.14	0.57	0.80	1.55	1.00	0.50	0.11	0.41	0.05	0.60
whitmorei									0.06			
Mansonia uniformis					0.05							
Tripteroides dyari				0.03	0.05			0.08				
nitidotenter					0.05							
Uranotaenia argyrotarsis												
atra			0.03									

TABLE 4.—*Monthly densities of culicine catches*—Continued.

Species	1955				Barrio Masiit*					1956		
	March	April	May	June	July	August	September	October	November	December	January	February
Aedes aegypti										0.05		
albopictus								0.05				
flaviventris								0.05				
lineatopennis								0.05		0.05		0.10
nocturnus								0.10				
pampangensis					0.10	0.10						
poicilius												
Armigeres baisasi					0.05		0.05					
Culex annulus								0.05		0.05	0.05	
bitaeniorhynchus	0.03											
fatigans	1.00	3.08	0.18	0.03	0.95	0.15	0.15	0.39	0.11	0.50	1.80	0.00
frandatrix												
fuscanus										0.05		
fuscocephalus										0.05		
gelidus							0.05		0.06	0.10		0.10
incognitus	0.10				0.05	0.10	0.14	0.17			0.05	
malayi-laureli							0.09					
rubithoracis												
summorosus			0.03		0.20	0.15	0.14	0.22		0.55	0.19	
whitmorei										0.05		
Mansonia uniformis									0.06			
Tripteroides dyari												
nitidocenter												
Uranotaenia argyrotarsis										0.05	0.05	
atra												

* Sprayed with DDT—February, 1955; February, 1956
Sprayed with DN—February, 1957.

albopictus highly preferred. Similarly, the higher *flavirostris* catches in Barrio San Antonio cannot be taken to gauge the effect of DDT against *flavirostris* in Barrio Masiit. Before DDT was ever used in Masiit and Calauan, expert workers like Russell, King, and others found very few or no *flavirostris* in houses of these places at the daytime. The few houses which gave high *flavirostris* catches in Barrio San Antonio were unusual for Laguna Province or for Luzon Island as a whole.

Pre-spraying observations should have been done. But that was not possible. By the time observations became at all feasible, Masiit houses had already received two consecutive yearly DDT sprayings. It was not also possible to undertake a simultaneous outdoor observation, at least regarding vector species and house pests due to lack of personnel.

Whether the effects of DDT against *Aedes aegypti* were purely toxic or both toxic and repellent could not be ascertained. Although none was caught in houses sprayed with DDT at Barrio Masiit, it could easily be caught biting outdoor.

Despite DDT, more *quinquefasciatus* were caught indoor in Barrio Masiit than in Barrio San Antonio. This might have indicated two things: (*1*) constantly higher *quinquefasciatus* population in Barrio Masiit; and (*2*) naturally high non-susceptibility of this mosquito to DDT. Its high and rapidly increasing resistance (in the presence of insecticides to DDT has long been known and repeatedly proved in other countries. Local *quinquefasciatus* seems to be the same as shown by numerous catches of this mosquito while resting on DDT-sprayed surfaces indoors both in Sorsogon Province, Luzon Island; and in Cotabato Province, Mindanao Island.

SUMMARY

1. The results of one year's observation in houses of Barrio Masiit, Calauan (DDT-sprayed), and Barrio San Antonio, San Pablo City (not treated with DDT), both of Laguna Province, Luzon Island, are presented. These areas were selected for contrast rather than for similarities.

2. An average of 0.964 female *flavirostris* per man, per hour was caught in Barrio San Antonio; the average for both sexes was 1.061. From the sprayed houses at Barrio Masiit, an average of 0.004 female *flavirostris* was captured; 0.013 for both sexes. This did not necessarily mean DDT had reduced indoor *flavirostris* that much in Barrio Masiit, compared with

the catches from Barrio San Antonio, since Masiit had long been known for the scarcity or absence of *flavirostris* in houses; whereas the high *flavirostris* catches in a few houses at Barrio San Antonio were unusual for Laguna Province or the entire Luzon Island.

3. Despite two consecutive yearly DDT-sprayings in Barrio Masiit, the average per man, per hour catch of *Culex quinquefasciatus* (0.879 for females; 1.248 for both sexes) was higher than those in Barrio San Antonio (0.391 for females; 0.610 for both sexes). This was probably the result of more extensive breeding areas in Masiit, and the naturally high (and rapidly increasing) resistance of *quinquefasciatus* to DDT.

4. House pest, *Aedes aegypti,* seemed to have been effectively eliminated from DDT-sprayed houses, although there is reason to believe that DDT also acted as repellent since *aegypti* was present outdoor.

5. Of the 91 different species and subspecies reported from these areas, representatives of only 42 were caught indoors.

6. *Quinquefasciatus* was the most often caught in DDT-sprayed houses of Masiit (densities shown in No. 4 above), followed by *Anopheles indefinitus* (densities: 0.286 for females; 0.412 for both sexes); then by *Anopheles limosus* (densities: 0.160 for females, 0.182 for both sexes). The highest catches from the non-sprayed houses of Barrio San Antonio were *Anopheles limosus* (densities: 2.667 for females, 2.714 for both sexes); *Anopheles indefinitus* (densities: 2.474 for females, 2.668 for both sexes); and *Anopheles minimus flavirostris* (see densities given above in Table 3).

7. Attempts were made to catch other insects of medical importance, but there were not many, and their identification will take a lot of time and study.[5]

8. The monthly densities of non-anophelines caught in houses during the observations are shown in Table 4.

9. 39 houses were positive for *flavirostris* in Barrio San Antonio, only 2 in Barrio Masiit; 20 for *mangyanus* in Barrio San Antonio, none in Barrio Masiit; 64 for *aegypti* in Barrio

[5] Among other insects found in houses were various species of: Blattidæ, Cecidomyidæ, Chironomidæ, Chloropidæ, Drosophilidæ, Ephemiridæ, Epydridæ Muscidæ, Mycetophilidæ, Psychodidæ, Sciaridæ, Sepsidæ, Stratiomyidæ, Tipulidæ; Hymenoptera and Lepidoptera.

San Antonio, none in Barrio Masiit. But while there were 46 houses positive for *quinquefasciatus* in Barrio San Antonio, there were 44 in Masiit; reduced to percentages, 48.93 per cent of the houses in barrio San Antonio, and 66.67 per cent in Barrio Masiit were positive for *quinquefasciatus*.

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MARINE FISHES IN PHILIPPINE RIVERS AND LAKES

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Most people like to go fishing. Whether they are young or old, male or female, doesn't seem to make much difference. And of course when they catch any fish they like to know something about them; whether they are good to eat, their food value, their habits and how they may be caught best, their distribution, time of year available, how they are best preserved for future consumption, etc.

I first went fishing in 1878 and have kept up my interest in fishes and fishing all through the intervening eighty years. All sorts of interesting things concerning fishes have been forced upon my attention, including problems far more difficult than merely catching and eating fish.

One of the most interesting of these problems is that of the distribution of fishes. Why do they occur in this place and not in that? Why do they migrate? Where do they come from? And where do they go when they leave our coasts?

One of the first things noticed about fishes was that some kinds were found only in salt water, others only in fresh water. Again some species live most of the time in the sea but enter fresh-water rivers and lakes to spawn, often going far inland for that purpose. On the other hand there are many fishes which enter fresh water soon after hatching, live there for a time, and finally return to the sea to spawn. This last group is prominent in the Philippines, and some of its members may ascend streams to a height of a thousand or even fifteen hundred meters above sea level before settling down.

In spite of these divergencies, the great majority of fishes in the cool and temperate waters of Europe, Asia, and North America are definitely either marine or fresh water, making little or no change of habitat during their lifetime. This is because most of them cannot endure much change in the salt content of the water surrounding them.

Fishes or other animals which can live through considerable changes of salinity are called euryhalin. Of course there are all degrees of euryhalinity; some can stand no change at all,

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others only a little. But there are many which can change from either salt to fresh water or the reverse, and can live in either kind indefinitely. In the old Manila aquarium we conducted many interesting experiments in transferring fish from salt to fresh water. Some fishes can make the change if it is done very slowly, but there are some which can swim directly from salt to fresh water or from a river into the sea, without any apparent injury at all.

Gunter defines a euryhalin fish as one which is recorded from both fresh and sea water, but in his list he includes fishes living in fresh water but derived from marine stock. Conversely he likewise includes fishes living in salt water but derived from fresh-water stock. This paper is an attempt to list such fishes as are known to fall under the above headings and are known to occur in Philippine waters. Fishes known only from the sea and brackish water are excluded, as are also strictly fresh-water fishes not of marine ancestry.

For a good many years I have been deeply interested in the fishes of Philippine rivers and lakes. It has been my privilege to observe them in the field from Aparri at the mouth of the Cagayan River on the north coast of Luzon to the rivers of the south coast of Mindanao, the Malum River of TawiTawi, and the creeks of Balabac. Not forgotten either were the lakes of Luzon, Mindoro, Negros, Mindanao, and the crater lakes of Sulu Province.

The student of Philippine fresh-water fishes is struck at once by the fact that over most of the islands the fishes of the rivers and lakes are either marine or else of marine origin. In the latter case they have taken up their abode in fresh water in recent time. Indeed some seem to be still in the process of making the change.

Only a very few families of true fresh-water fishes are native to the Philippines, as I showed in various papers long ago. They are all confined with one exception to the waters of those islands which were once connected via Borneo and Sunda Land with the mainland of Asia. Mindanao was connected via Basilan and the Sulu Archipelago with Borneo. Balabac, Palawan, and the Calamianes were connected by a land bridge with Borneo, via Banguey and Balambangan. By these two land bridges a few members of the cyprinidæ or carp family, and several species of two families of catfishes, the Clariidæ and the Siluridæ, were able to establish themselves in the islands

named above. To the islands named must be added Mindoro, where one species of cyprinid was able to come across from Busuanga. From the rest of the Philippines, these true fresh-water fishes were cut off by deep seas and swift currents.

All over the Philippines we find the well known climbing perch, *Anabas testudineus,* called liwalo in Tagalog and puyo in Bisayan, at home in lowland fresh waters. The dalag or haruan, *Ophicephalus striatus* is even more widespread as it is found from the lowlands to mountain lakes at an altitude of 1,000 meters. Both of these valued foodfishes were widely distributed by man, both in prehistoric and recent time. It is highly improbable that either one of them was originally native to the Bisayas or Luzon.

The following true fresh-water fishes now widely distributed in the Philippines are all of recent introduction, in the years between 1908 and 1938. Carp, *Cyprinus carpio,* from China by Alvin Seale, my predecessor, and again by me and still later by H. R. Montalban from Formosa. Gurami, *Osphronemus goramy,* from Java, by me. Sipat Siam, *Trichogaster pectoralis,* from Siam or Thailand, by Dr. Eduardo Quisumbing. The North American game fish, black bass, *Huro floridana,* now established in Lake Lanao and some of its tributaries, was introduced by Alvin Seale in 1908 but was confined to a small pool at Baguio till I planted it in the Taraka River in 1926.

There is one other fresh-water fish native to Luzon. It is a tiny obscure cyprinodont fish only known from a small area in Ilokos Norte Province, *Oryzias luzonensis.* It was probably introduced from China or Japan a long time ago and became modified in its new habitat.

The fact that most of the fishes of Philippine rivers and lakes are marine, and spend but a part of their lives in fresh water, gives rise to important fisheries in certain regions. As examples one may cite the catch of ipon or goby fry off the mouths of rivers in northern Luzon, or just within the river mouth. From the Cagayan River at Aparri across the north end of Luzon and down the west coast of the Ilokano provinces the catch of goby fry is of great importance at the mouth of every river. Goby fry or ipon are the raw material for the important *bagoong* industry. They are caught as they start their migration to the upland streams, but enough are allowed to escape to keep up the population in the interior rivers. When

the fish are adult they return to the sea, or in some species to brackish water at or near the river mouth, to spawn.

Other fisheries depending upon the migration of adult fish from lakes and rivers to the sea to spawn are: (*1*) the great baklad fisheries in the Pansipit River and in Lake Bombon; (*2*) the fisheries at the Butas baklad in the outlet of Lake Naujan and at the mouth of the river; (*3*) the catch of giñgao, *Lutianus argentimaculatus*, in rivers of the Bisayas and Mindanao; (*4*) the fishery at Jabonga, at the outlet of Lake Mainit.

The list of euryhalin fishes known from Philippine waters presented in this paper is not absolutely complete. It merely represents our present state of knowledge. As a matter of fact we do not have a complete list of the fishes living in fresh water for a single one of the Philippines with several streams. This knowledge will not be attained until every stream in each island has been explored from the sea to its source. Where this has been done surprising results have been obtained, and our knowledge of the distribution of Philippine fishes has been greatly increased. When a survey has been made of every stream in the Philippines it is certain that fishes as yet known only from the sea will be found in fresh water. This will be especially true when the highly oxygenated mountain streams and the river rapids have been thoroughly worked.

Euryhalin fishes are usually divided by North American writers into anadromous and catadromous fishes. Anadromous fishes are those which spend most of their lives in the sea but must enter fresh water to breed.

In his "Revised List of Euryhalin Fishes of North and Middle America," Gunter states on page 351: "All anadromous species are found in the temperate zone and northward. None is known from the tropics." He has overlooked the species of *Hilsa*, shadlike clupeoid fish which ascend the rivers of China and India to spawn. The Indian rivers are certainly in the tropics and their *Hilsa* fisheries are still of some importance in spite of the efforts of irrigation engineers and other dam builders to destroy them by cutting the fish off from their spawning grounds.

The salmon of the North Pacific and North Atlantic are also notable examples of fish which must return to fresh water to spawn. We have no anadromous fishes in the Philippines. All our euryhalin fishes are catadromous; that is they must migrate to brackish or salt water to breed after spending most of their

life in fresh water. It is possible that some of those listed are able to breed in fresh water at time. A large number of fishes in the Philippines merely visit fresh water for a time, entering while quite young and staying for a longer or shorter time but returning to the sea long before sexual maturity and therefore not under the compulsory urge of the need to spawn.

List A contains marine fishes definitely recorded from fresh water in the Philippines.

List B contains marine fishes known to occur in fresh water but without a definite record in a Philippine river or lake, although they are known from the Philippines. Fishes occurring in fresh water in neighboring islands, such as Borneo, but never recorded from the Philippines, are not listed here even though it is almost certain that they do occur in Mindanao or Palawan.

List C comprises those fishes of marine origin now living and breeding entirely in fresh water, as far as is known.

List D includes the few fishes of marine origin which live and breed in fresh water but which may enter brackish and salt water during the rainy season, but which do not breed there so far as known.

List E is for an introduced and naturalized fish which enters salt water and apparently breeds freely in fresh, brackish, and salt water.

It is possible that some of the Eleotridæ in List A may breed in fresh water as well as in salt or brackish water. The same remark holds true of some of the gobies.

LIST A

Marine fishes returning to the sea or to brackish water to spawn.

CLASS ELASMOBRANCHII

Pating; pagi; sharks; rays

Subclass SELACHII

Pating; sharks

Family ORECTOLOBIIDÆ

1. *Stegastoma varium* (Seba). Butanding; zebra shark.

Family CARCHARIIDÆ

2. *Carcharias gangeticus* Müller and Henle. Pating; ganges shark.

Order BATOIDEI

Family PRISTIDÆ

3. *Pristis microdon* Latham. Tangan; sawfish.

CLASS PISCES

True fishes

Order ISOSPONDYLI

Family ELOPIDÆ

4. *Megalops cyprinoides* (Broussonet). Buan-buan; small tarpon.

Family CHANIDÆ

5. *Chanos chanos* (Forskål). Bañgos.

Family DUSSUMERIIDÆ

6. *Etrumeus albulina* Fowler.

Family DOROSOMATIDÆ

7. *Anodontostoma chacunda* (Hamilton). Kabasi.
8. *Nematalosa nasus* (Bloch). Kabasi; suagan.

Family CLUPEIDÆ

9. *Sardinella fimbriata* (Cuv. and Val.). Tamban; tunsoy; sardine.
10. *Harengula punctata* (Rüppell). Tunsoy; Sardine.

Family ENGRAULIDÆ

11. *Thrissina baelama* (Forskål). Dumpilas; anchovy.
12. *Stolephorus commersoni* Lac. Dilis: long-jawed anchovy.
13. *Stolephorus indicus* (van Hasselt). Tuakang; dilis.

Family ANGUILLIDÆ

14. *Anguilla celebesensis* Kaup. Kasili; celebes eel.
15. *Anguilla marmorata* Quoy and Gaim. Igat; kasili; marbled eel.
16. *Anguilla pacifica* Schmidt. Igat; kasili.
17. *Anguilla spengeli* Weber. Kasili; Spengel's eel.

Family MURÆNESOCIDÆ

18. *Muraenesox cinereus* (Forskål). Pindañga; pike eel.

Family OPHICHTHYIDÆ

19. *Achirophichthys kampeni* (Weber and DeBeaufort).
20. *Cæcula kaupi* (Bleeker).
21. *Cæcula mindora* (Jordan and Richardson).
22. *Cæcula taylori* Herre.
23. *Lamnostoma polyophthalmus* (Bleeker).
24. *Pisoodonophis boro* (Hamilton).

Family MURÆNIDÆ

Morays

25. *Echidna rhodochilus* Bleeker.
26. *Evenchelys macrurus* (Bleeker).
27. *Gymnothorax meleagris* (Shaw).
28. *Gymnothorax polyuranodon* (Bleeker).

Order SYNBRANCHIA

Family FLUTIDÆ

29. *Synbranchus bengalensis* (McClell.) Tale-rek; rice field eel.

Order NEMATOGNATHI

Family ARIIDÆ

Catfishes

30. *Arius crossocheilus* Bleeker.
31. *Arius thalassinus* (Rüppell).

Order SYNENOGNATHI

Family HEMIRAMPHIDÆ

Half-beaks

32. *Hemiramphus gaimardi* Cuv. and val. Susay.
33. *Hemiramphus marginatus* (Forskål). Soacid.
34. *Hemiramphus quoyi* Cuv. and val. Salasa.
35. *Hemiramphus unifasciatus* Ranzani.
36. *Zenarchopterus brevirostris* (Günther).
37. *Zenarchopterus buffoni* (Cuv. and Val.).
38. *Zenarchopterus dispar* (Cuv. and Val.).
39. *Zenarchopterus kampeni* Weber.

Order HETEROSOMATA

Family CYNOGLOSSIDÆ

40. *Cynoglossus puncticeps* (Rich.). Dapañg sinilas; speckled sole.

Order BERYCOIDEI

Family HOLOCENTRIDÆ

Soldier fishes

41. *Holocentrus cornutus* Bleeker. Sugak.

Order THORACOSTEI

Suborder LOPHOBRANCHII

Family SYNGNATHIDÆ

Pipe-fishes

42. *Belonichthys fluviatilis* (Peters).
43. *Bombonia djarong* (Bleeker).
44. *Bombonia spicifer* (Rüppell).
45. *Coelonotus leiaspis* (Bleeker).
46. *Doryichthys retzi* (Bleeker).
47. *Oostethus brachyurus* (Bleeker).
48. *Oostethus manadensis* (Bleeker).
49. *Syngnathus cyanospilus* Bleeker.

Order PERCOMORPHI

Family ATHERINIDÆ

Guno; hardheads

50. *Atherina endrachtensis* Quoy and Gaimard.
51. *Hepsetia balabacensis* Seale.

Family MUGILIDÆ

Banak; Mullet

52. *Mugil banksi* Seale.
53. *Mugil cephalus* Linnaeus.
54. *Mugil cunnesius* Cuv. and Val.
55. *Mugil dussumieri* Cuv. and Val. Aligasin: aliso; banak.
56. *Mugil engeli* Bleeker.
57. *Mugil longimanus* Günther.
58. *Mugil tade* (Forskål).
59. *Liza caeruleo-maculata* (Lac.).
60. *Liza ceramensis* (Bleeker).
61. *Liza macrolepis* (Smith). Aguas.
62. *Liza melinoptera* (Cuv. and Val.). Talilong.
63. *Liza seheli* (Forskål).
64. *Liza vaigiensis* (Quoy and Gaimard).
65. *Cestraeus goldei* (Macleay).

Suborder PHALLOSTETHOIDEA

Family PHALLOSTETHIDÆ

66. *Ceratostethus bicornis* (Regan).
67. *Neostethus amaricola* (Villadolid and Manacop).
68. *Neostethus (Sandakanus) coronensis* Herre.

Suborder RHEGNOPTERI

Family CARANGIDÆ

Talakitok; momsa; jacks

69. *Alectis indica* (Rüppell).
70. *Caranx carangus* (Bloch).
71. *Caranx dinema* (Bleeker).
72. *Caranx ignobilis* (Forskål). Malipitó; sumañga.
73. *Caranx sexfasciatus* Quoy and Gaim. Muslo; pinkit; simbad.
74. *Caranx stellatus* Eydoux and Souleyet.
75. *Citula armata* (Forskål). Ama-aligan; buensañg-sapse.
76. *Megalaspis cordyla* (L.) Adlao; oriles; hardtail.
77. *Seriola nigrofasciata* (Rüppell).

Family LEIOGNATHIDÆ

Sap-sap; slip-mouth

78. *Leiognathus dussumieri* (Cuv. and Val.). Malaway; sapsap.
79. *Leiognathus equulus* (Forskål). Lawayan; malaway; sapsap.
80. *Leiognathus splendens* (Cuvier). Masañgi.

Family GERRIDÆ

81. *Gerres abbreviatus* Bleeker. Malakapas; bansa.
82. *Gerres filamentosus* Cuvier. Hamarok; manobon.
83. *Gerres macracanthus* Bleeker. Big-spine malakapas.
84. *Gerres oyena* (Forskål).
85. *Gerres poieti* Cuvier.

Family APOGONIDÆ

Beñgka dañgat; cardinal fishes

86. *Apogon amboinensis* Bleeker. Matan; parañgan.
87. *Apogon diversus* (Smith and Radcliffe).
88. *Apogon hyalosoma* Bleeker.
89. *Apogon lateralis* Valenciennes.

Family AMBASSIDÆ

Lañgaray; glass fish

90. *Ambassis buruensis* Bleeker.
91. *Ambassis commersoni* (Cuv. and Val.).
92. *Ambassis gymnocephalus* (Lacépède).
93. *Ambassis interrupta* Bleeker.
94. *Ambassis kopsi* Bleeker.

95. *Ambassis miops* Günther.
96. *Ambassis nalua* (Hamilton).
97. *Ambassis urotaenia* Bleeker.

Family SERRANIDÆ

Lapo-lapo; sea bass; grouper

98. *Lates calcarifer* (Bloch). Apahap; white sea bass.
99. *Psammoperca waigiensis* (Cuv. and Val.).
100. *Cephalopholis miniatus* (Forskål).
101. *Epinephelus malabaricus* (Bl. and Schn.). Malabar lapo-lapo.
102. *Epinephelus tauvina* (Forskål). Kulapo; kugtung.
103. *Cromileptes altivelis* (Cuv. and Val.). Lapo-lapoñg manochot.

Family LOBOTIDÆ

104. *Lobotes surinamensis* (Bloch). Kakap bato.

Family LUTIANIDÆ

Snappers

105. *Lutianus argentimaculatus* (Forskål). Also; Giñgao; gray snapper.
106. *Lutianus biguttatus* (Cuv. and Val.). Two-spot snapper.
107. *Lutianus bohar* (Forskål). Maya-maya; bohar red snapper.
108. *Lutianus chrysotaenia* (Bleeker). Golden-banded snapper.
109. *Lutianus decussatus* (Cuv. and Val.). Dolesan; checkered snapper.
110. *Lutianus fulviflamma* (Forskål). Bahaba; gold flame snapper.
111. *Lutianus fuscescens* (Cuv. and Val.). Kamañgbuhu.
112. *Lutianus johni* (Bloch). Bitilla; maya-maya.
113. *Lutianus malabaricus* (Bl. and Schn.) Maya-maya; bakba-an.
114. *Lutianus maxweberi* Popta.
115. *Lutianus vaigiensis* (Quoy and Gaimard).

Family POMADASYIDÆ

Grunts

116. *Plectorhinchus chaetodonoides* (Lac.).
117. *Plectorhinchus crassispina* (Rüppell).
118. *Pomadasys hasta* (Bloch). Aguut; silver-spotted grunt.
119. *Scolopsis ciliatus* (Lac.).

Family THERAPONIDÆ

120. *Therapon argenteus* (Cuv. and Val.). Arad-ad; silver therapon.
121. *Therapon cancellatus* (Cuv. and Val.). Bagaong; cross-barred grunt.
122. *Therapon jarbua* (Forskål). Goñgong; Bagaoñg; malabansi.
123. *Pelates quadrilineatus* (Bloch). Agaak; Dahosan; four-barred grunt.

Family LETHRINIDÆ

124. *Lethrinus miniatus* (Forster). Kutambak; long-snouted kutambak.
125. *Lethrinus nebulosus* (Forskål). Kirawan; pearl-spotted kutambak
126. *Lethrinus rhodopterus* Bleeker. Bakutut.

Family SPARIDÆ

127. *Sparus berda* Forskål. Bakoko; porgy.

Family MULLIDÆ

Balaki; tiao; timbuñgan; goat-fishes

128. *Parupeneus indicus* (Shaw).
129. *Parupeneus pleurospilos* (Bleeker).
130. *Upeneus moluccensis* (Bleeker).
131. *Upeneus sulphureus* (Cuv. and Val.).
132. *Upeneus tragula* Richardson.
133. *Upeneus vittatus* (Forskål).

Family SILLAGINIDÆ

Asohos

134. *Sillago sihama* (Forskål).

Family MONODACTYLIDÆ

135. *Monodactylus argenteus* (L.). Muray-buray; silver batfish.

Family PLATACIDÆ

136. *Platax orbicularis* (Forskål). Dahon gabi; leaf-fish.

Family DREPANIDÆ

137. *Drepane punctata* (L.). Bayang; ariring; spotted drepane.

Family TOXOTIDÆ

Ataba; isdang sumpit; archer fish

138. *Toxotes jaculator* (Pallas).
139. *Toxotes chatareus* (Hamilton).
140. *Toxotes oligolepis* Bleeker.

Family SCATOPHAGIDÆ

141. *Scatophagus argus* (L.). Kitang; spadefish.
142. *Scatophagus tetracanthus* (Lac.).

Family CHÆTODONTIDÆ

143. *Heniochus acuminatus* (L.). Kabubu; white-plumed kabubu.

Family ACANTHURIDÆ

Labahita; surgeon fishes

144. *Acanthurus nigrofuscus* (Forskål).
145. *Acanthurus philippinus* Herre.
146. *Zebrasoma veliferum* (Bloch).

Family TEUTHIDIDÆ

147. *Teuthis concatenata* (Cuv. and Val.). Samaral.
148. *Teuthis fuscescens* (Houttuyn). Palit.
149. *Teuthis javus* Linnæus.
150. *Teuthis oramin* (Bl. and Schn.). Titang.
151. *Teuthis vermiculatus* (Cuv. and Val.).
152. *Teuthi virgata* (Cuv. and Val.). Mandalada.

Order CATAPHRACTI

Family SCORPÆNIDÆ

153. *Gymnapistes niger* (Cuv. and Val.). Black sea wasp.
154. *Tetraroge barbata* (Cuv. and Val.).

Order CHROMIDES

Family POMACENTRIDÆ

Tibuk; aroro baybay coral fishes; damsel fishes

155. *Abudefduf azurepunctatus* Fowler and Bean.
156. *Abudefduf bengalensis* (Bloch).
157. *Abudefduf melas* (Cuv. and Val.).
158. *Pomacentrus littoralis* Cuv. and Val.
159. *Pomacentrus perspicillatus* Cuv. and Val.
160. *Pomacentrus prosopotaenia* Bleeker.
161. *Pomacentrus taeniurus* Bleeker.
162. *Pomacentrus tripunctatus* Cuv. and Val.

Family LABRIDÆ

163. *Cheilinus diagrammus* (Lac.).
164. *Xiphocheilus quadrimaculatus* Günther.

Family SCARIDÆ

Mulmul; ogos; parrot fish

165. *Scarus dimidiatus* Bleeker.
166. *Scarus dubius* Bennett.
167. *Scarus oedema* (Snyder).
168. *Scarus sordidus* Forskål.

Order GOBIOIDEA

Family RHYACICHTHYIDÆ

169. *Rhyacichthys aspro* (Cuv. and Val.) Kampa; delaposkan; pilingan.

Family ELEOTRIDÆ

170. *Belobranchus belobranchus* (Cuv. and Val.).
171. *Boroda albo-oculata* Herre.
172. *Boroda francoi* Roxas and Ablan.
173. *Bunaka pinguis* Herre. Bunak

174. *Bunaka sticta* Herre. Spotted bunak.
175. *Butis amboinensis* (Bleeker).
176. *Butis butis* Hamilton.
177. *Butis gymnopomus* (Bleeker).
178. *Eleotris fusca* (Bl. and Schn.). Lamug.
179. *Eleotris melanosoma* Bleeker. Virot.
180. *Hypseleotris modestus* (Bleeker). Lomog.
181. *Hypseleotris pangel* Herre. Pañgel.
182. *Odontobutis obscura* (Schlegel).
183. *Ophiocara aporos* (Bleeker). Bañgañgay,
184. *Ophiocara porocephala* (Cuv. and Val.). Palu; paku
185. *Paloa polylepis* Herre.
186. *Paloa villadolidi* Roxas and Ablan.
187. *Parviparma straminea* Herre.
188. *Prionobutis koilomatodon* (Bleeker).

Family GOBIIDÆ

Bia; goby

189. *Amblygobius decussatus* (Bleeker).
190. *Apocryptodon glyphisodon* (Bleeker).
191. *Brachygobius aggregatus* Herre.
192. *Chonophorus genivittatus* (Cuv. and Val.).
193. *Chonophorus lachrymosus* (Peters). Biañg paku; biañg tulis.
194. *Chonophorus melanocephalus* (Bleeker): Bukto; bunog; biañg bato.
195. *Chonophorus ocellatus* (Broussonet).
196. *Creisson janthinopterus* (Bleeker).
197. *Ctenogobius caninus* (Cuv. and Val.).
198. *Ctenogobius criniger* (Cuv. and Val.) Lañginlagen.
199. *Glossogobius biocellatus* (Cuv. and Val.). Biañg tulog; mulug.
200. *Glossogobius celebius* (Cuv. and Val.). Bal-la; biañg bato; rock goby.
201. *Glossogobius giurus* ((Hamilton). Biañg puti; White goby.
202. *Gnatholepis puntangoides* (Bleeker). Burok; Kamumbon.
203. *Illana bicirrhosa* (Weber).
204. *Oligolepis acutipennis* (Cuv. and Val.).
205. *Oxyurichthys microlepis* (Bleeker).
206. *Oxyurichthys ophthalmonema* (Bleeker).
207. *Schismatogobius bruynisi* De Beaufort.
208. *Schismatogobius insignum* (Herre).
209. *Schismatogobius marmoratus* (Peters).
210. *Schismatogobius roxasi* Herre.
211. *Sicyopterus crassus* Herre.
212. *Sicyopterus cynocephalus* (Cuv. and Val.).
213. *Sicyopterus extraneus* Herre.
214. *Sicyopterus fuliag* Herre.
215. *Sicyopterus lacrymosus* Peters. Paliling.
216. *Sicyopterus panayensis* Herre.
217. *Sicyopus zosterophorum* (Bleeker).
218. *Stiphodon elegans* (Steindachner).

Seems to breed in fresh, brackish, and salt water.

219. *Stiphodon formosus* (Herre).
220. *Vaimosa bikolana* Herre.
221. *Vaimosa cardonensis* Herre.
222. *Vaimosa macrognathos* Herre.
223. *Vaimosa piapensis* Herre.
224. *Vaimosa rivalis* Herre.
225. *Vaimosa sapanga* Herre.

Family GOBIOIDIDÆ

226. *Caragobius typhlops* Smith and Seale.
227. *Taenioides caeculus* (Bl. and Schn.). Baliga.
228. *Taenioides cirratus* (Blyth).
229. *Taenioides gracilis* (Cuv. and Val.).

Order JUGULARES

Family CALLIONYMIDÆ

230. *Callionymus calauropomus* Richardson.

Family TRICHONOTIDÆ

231. *Gobitrichonotus radiocularis* Fowler.
232. *Trichonotus setiger* Bl. and Schn.

Family BLENNIIDÆ

233. *Petroscirtes feliciana* Herre.

Family TETRAODONTIDÆ

234. *Chelonodon patoca* (Hamilton).

LIST B

Marine fishes known to occur in fresh water but with no exact and definite Philippine record of such occurrence.

Family DASYATIDÆ

Pagi: sting ray

The early chronicles of the Spanish friars told of a ray in Lake Naujan. They wrote in detail of how the native caught gravid female rays, removed the very large eggs which were highly esteemed, and threw the fish back in the lake. We have no way of knowing what this ray was, and on my trips to the lake I was never able to get a specimen.

At Moncayo, on the upper Agusan River, the people reported to me that they caught rays there. They also reported the Ganges shark and sawfish. A sketch of a ray was made but

one could not tell the exact species from it. The Agusan River ray is certainly one of the two species following and the Lake Naujan ray is very likely one of them.

1. *Dasyatis kuhli* (Müller and Henle).
2. *Dasyatis uarnak* (Forskal).

Family CLUPEIDÆ

3. *Sardinella brachysoma* Bleeker.
4. *Sardinella longiceps* Cuv. and Val. — Tamban; sardine.
5. *Harengula dispilonotus* Bleeker.

Family ENGRAULIDÆ

6. *Thrissocles kammalensis* (Bleeker).
7. *Scutengraulis mystax* (Bl. and Schn.).
8. *Stolephorus tri* Bleeker.

Family MURÆNIDÆ

9. *Gymnothorax tile* (Hamilton).

Family ARIIDÆ

10. *Arius sagor* (Hamilton).

Family PLOTOSIDÆ

11. *Plotosus anguillaris* (Bloch).

Family HEMIRAMPHIDÆ

12. *Dermogenys pusillus* Van Hasselt.

Family MUGILIDÆ

13. *Mugil subviridis* Cuv. and Val.
14. *Liza oligolepis* (Bleeker).
15. *Cestraeus oxyrhynchus* Cuv. and Val.

Family LEIOGNATHIDÆ

16. *Leiognathus insidiator* (Bloch). — Dalupane; bilong-bilong.
17. *Leiognathus ruconius* (Hamilton). — E-im.

Family APOGONIDÆ

18. *Apogon compressus* (Smith and Radcliffe).
19. *Apogon frenatus* Valenciennes.

Family SERRANIDÆ

20. *Epinephelus corallicola* (Cuv. and Val.)

Family LUTIANIDÆ

21. *Lutianus gibbus* (Forskål). Maya-maya; red snapper.
22. *Lutianus russelli* (Bleeker).

Family POMADASYIDÆ

23. *Plectorhinchus schotaf* (Forskål).
24. *Scolopsis vosmeri* (Bloch).

Family THERAPONIDÆ

25. *Therapon puta* Cuv. and Val. Babansi; grunt.

Family NEMIPTERIDÆ

26. *Nemipterus oveniides* (Popta).

Family SCIÆNIDÆ

27. *Johnius belengeri* (Cuv. and Val.). Ibot; croaker.
28. *Pseudosciaena aneus* (Bloch). Alaka-ak

Family POMACENTRIDÆ

29. *Pomacentrus melanopterus* Bleeker.

Family ELEOTRIDÆ

30. *Bostrichthys sinensis* (Lacépède).

Family GOBIIDÆ

31. *Gobius oligolepis* Bleeker.
32. *Stigmatogobius javanicus* (Bleeker).
33. *Stigmatogobius tambujon* (Bleeker).

Family TETRAODONTIDÆ

34. *Tetraodon fluviatilis* Hamilton.

LIST C

Fishes of marine origin now living and breeding in fresh water, as far as is known, and not going to brackish or salt water.

Family CLUPEIDÆ

1. *Harengula tawilis* Herre. Tawilis; Lake Bombon sardine.

Family FLUTIDÆ

2. *Fluta alba* (Zuiew). Rice field eel.

Family ARIIDÆ

3. *Arius magatensis* Herre.
4. *Hemipimelodus manillensis* (Cuv. and Val.).

Family HEMIRAMPHIDÆ

5. *Zenarchopterus cagayensis* Herre.
6. *Zenarchopterus* cotnog H. M. Smith. Kotnog.
7. *Zenarchopterus philippinus* Peters.
8. *Zenarchopterus philippinus* var. *magatensis* Herre.
9. *Dermogenys viviparus* Peters.

Family SYNGNATHIDÆ

10. *Doryichthys pleurostictus* (Peters).

Family PHALLOSTETHIDÆ

11. *Ctenophallus ctenophorus* (Aurich).
12. *Gulaphallus eximius* Herre.
13. *Gulaphallus mirabilis* Herre.
14. *Manacopus falcifer* (Manacop).
15. *Mirophallus bikolanus* Herre.
16. *Solenophallus thessa* Aurich.

Family APOGONIDÆ

17. *Mionurus bombonensis* Herre.

Family KUHLIIDÆ

18. *Kuhlia marginata* (Cuv. and Val.). Damagan.
19. *Kuhlia rupestris* (Lac.). Kalabingat.

Family THERAPONIDÆ

20. *Datnia plumbea* Kner. Ayungin.

Family ELEOTRIDÆ

21. *Boroda expatria* Herre.
22. *Hypseleotris agilis* Herre.
23. *Hypseleotris bipartita* Herre.

Family GOBIIDÆ

24. *Gnatholepis volcanus* Herre.
25. *Mirogobius lacustris* Herre.
26. *Mirogobius stellatus* Herre.
27. *Mistichthys luzonensis* H. M. Smith.
28. *Rhinogobius bucculentus* (Herre).
29. *Rhinogobius carpenteri* Seale.
30. *Rhinogobius philippinus* (Herre). Biang tuku; lizard goby.
31. *Tamanka cagayanensis* (Aurich).
32. *Tamanka maculata* Aurich.
33. *Tamanka siitensis* Herre.
34. *Vaimosa dispar* (Peters). Irin-irin.
35. *Vaimosa montalbani* Herre.

Family BLENNIIDÆ

36. *Petroscirtes ferox* Herre.

LIST D

Fishes of marine origin now living and breeding in fresh water but known to enter brackish and salt water at times.

Family ARIIDÆ

1. *Arius dispar* Herre.
2. *Arius manillensis* Cuv. and Val.

LIST E

Fresh-water fish entering salt water and apparently breeding freely in fresh, brackish, and salt water.

Family PŒCILIIDÆ

1. *Mollienesia latipinna* Le Sueur. Bubuntis; sailfin.

Accidentally introduced into the Philippines from Honolulu, where it was in turn introduced from the coast of Texas. Now naturalized around Manila Bay and swarming in fish ponds and salt ponds. Strangely enough, it shows a greater tolerance for salt than any native fish and is the last one to succumb in the salt ponds. It is still active when all other fish have been killed.

REMARKS

The combined lists contain 307 species, a number more than twice that known from the entire American continent north of Panama.

The order Gobioidea has the largest number of euryhalin fishes in the Philippine fauna. One member of the Rhyacichthyidæ, nineteen species of Eleotridæ, thirty-six of the Gobiidæ, and four species of Gobioididæ are known from fresh water but do not breed there. These all enter fresh water from the sea or brackish water and remain there until adult or nearly so. In addition to these, there are three species of Elotridæ and twelve species of Gobiidæ which live entirely in fresh water as far as is now known. This is a total of 75 species. There are several other Philippine gobies which are known to live in fresh water elsewhere, but for which there are no Philippine records of their occurrence in a stream or lake.

In the goby genus *sicyopterus* with one species, *Sicyopterus extraneus*, descends to the vicinity of the sea but lays its eggs in fresh water. The larvæ go on down to salt water and then migrate up stream to the interior of Mindanao. However in the rivers of northern Luzon other species of the same genus

descend to the sea and lay their eggs off shore within a few kilometers of the river mouth. The ecological conditions are very different on the Ilokano and Aparri coasts from those at the mouth of the Cagayan River on the north coast of Luzon. After hatching the almost colorless transparent scaleless larvæ, but 10 or 12 mm in length, swarm toward the river mouth. Immense numbers are taken in nets then and in traps later in the river mouth itself.

Other marine groups which are strongly represented in rivers and lakes are the mullets, family Mugilidæ, with 14 species definitely recorded; the Ambassidæ with 8 species; the Lutianidæ with 11 species; the Carangidæ with 9 species. Only one lutianid, *Lutianus argenti-maculatus*, enters lakes and rivers in any considerable numbers.

Only two species of Carangidæ enter Philippine fresh waters in numbers sufficient to be of importance. *Caranx sex-fasciatus* Q. and G. enters fresh water in considerable numbers while quite small, from about 25 to 40 or 45 mm in length. They do not go in schools but singly, and in favorable places, as at the Pansipit River baklad a steady stream of them may be seen going up stream. At the same time one may observe a good many of the same species larger in size, from 75 to 150 mm in length, making their way down stream in a steady dribble. Later on, during the dry season, considerable numbers of this species migrate toward the sea and are caught in quantity in the baklad or fish corrals in the outlet of the lake. These fish are scarcely ever more than half grown, and the urge to spawn is certainly not their driving force. They are probably not over a year old and may be less, and this species never remains in fresh water till it approaches maturity. These migrating fish are about 300 to 400 mm in length. My estimate of their age is based upon experiments with young jacks in the Manila Aquarium. Fish not over 25 mm in length grew to a length of 600 to 700 mm in two years.

The other species, formerly occurring in considerable numbers in Laguna Bombon and Lake Naujan is *Caranx ignobilis*. When this species enters fresh-water lakes it remains there until it is almost sexually mature. Its driving force for seaward migration is the urge to spawn. Formerly specimens 750 mm to a meter and more in length, and very bulky, were common. But no fish of such size could escape the baklad, and during the last thirty years the number of this species taken from fresh water has dwindled to almost nothing.

While most fishes entering rivers from the sea are small and very young, the adults of some marine species freely enter fresh water. *Toxotes jaculator,* the archer fish or isdang sumpit, may be seen swimming at the surface and going up some stream with the incoming tide. Often a group of them keeps on traveling till far beyond the influence of salt water, anywhere from 5 to 40 kilometers. In the rainy season schools of glass fish, family Ambassidæ, swarm up the Pasig River, the Pansipit, and the Butas to the lakes from which these rivers flow. Young-glass fish ascend too but are not so conspicuous. Then in the dry season schools of glass fish go swirling down to the sea to spawn. They can be seen very easily as they cross the clear shallow rapids of the Butas River.

Juvenile mullet, family Mugilidæ, perhaps 20 to 45 mm in length, crowd up stream and ascend to the high plateaus of Luzon and Mindanao, 3,000 feet and more above the sea. If there is no waterfall to stop them they may ascend as far as gobies and eels, which in some cases reach a height of 5,000 feet. Mullet remain in Laguna de Bay, Laguna Bombon, and Lake Naujan, or the streams of the Bukidnon tableland or the heights of Sagada till almost ready to spawn, their gonads gorged. In the rainy season they rush for the sea and are caught in large numbers.

The most important single fish in the Philippines, bañgos (*Chanos chanos*) also enter streams and ascend to lakes of slight altitude when they are available. There they remain until they are about 750 mm or more in standard length, and are in their third year. Then they start for the sea but nearly all leaving Lake Bombon and Lake Naujan are taken in the fish corrals in the lake outlets. Formerly all were taken but in recent years provision has been made for the escape of a portion of the fish.

It is well to call attention to the fact that only a small part of the total population of fry of a given species of mullet, *Caranx, Lutianus,* or of bañgos ever enters fresh water. This is true also of some of the gobies, as *Glossogobius giurus,* which may be seen spending their whole life on some atoll or reef while others of the same species migrate to the interior of Mindanao, Luzon, etc. So too the fries of the black-finned mullet, *Liza waigiensis,* are very numerous on the strand of some coral isles which have no fresh water except when it rains. The well known presence of this species in fresh water was accidentally

omitted from my Check List. So too with *Caranx sexfasciatus* and *Lutianus argenti-maculatus;* in spite of the numbers entering fresh water, the bulk of the total population never enters fresh water. This likewise holds true for the Ganges shark, which may swarm and breed in the lagoon of some South Pacific atoll a vast distance from a river. Only a small part of the countless millions of bañgos fry which appear at shallow sandy beaches ever enter a fresh-water stream.

This invalidates the argument that all euryhalin fish need to visit fresh water at some period of life other than the breeding period. Those which find a stream or lake at hand certainly thrive there very well, especially in plankton rich lakes like Naujan. That such a journey is obligatory is disproven by the great numbers of the same species which never leave salt water.

Yet the statement does hold true for certain fishes. Conspicuous examples are found in some of the gobioid fishes as well as in the case of the igat or eel, *Anguilla* species. For the fry or larval stage of such fishes to enter fresh water and live there until adult or almost sexually mature is indeed compulsory. And for most of such species it is equally compulsory for them to descend to brackish or salt water to spawn.

There are a few fishes whose life history demands clarification, since they seem to breed in salt, brackish, or fresh water. At the same time there is no absolute proof that they do. One of these fish is *Toxotes jaculator,* the archer fish or *Isdang sumpit.* It may breed in fresh water at times but no one has any proof of that. Another is *Tamanka siitensis,* which I discovered in crater lakes on the Island of Jolo. These lakes are fresh water and have neither inlet nor outlet. I also found it in small streams on the north coast of Lanao Province, Mindanao. It certainly breeds in the Jolo lakes but does it do so in the Titunod River of Lanao? Or does it go to the sea less than 5 kilometers away? These are not the only fishes to be investigated but are merely cited as examples.

One reason for the large number of gobies entering the fresh waters of Luzon and the Bisayan islands is that these regions lacked true fresh-water fishes. The great variety of carps characteristic of Asian and eastern North American waters is entirely lacking. To fill this ecological vacancy various marine fishes, but especially the gobies, took over the functions elsewhere fulfilled by the carps.

The Ariidæ are given by authors as fishes living in salt water but derived from fresh-water stock. This statement does not hold true for the species of *Arius* in Luzon which live and breed in fresh water. This is shown by the geological history of Luzon. This large island, formed by the coalescence of smaller islands, has never had any connection with the mainland of Asia, but has always been isolated and surrounded by deep seas. The sea is therefore the only place from which the Ariid catfishes in its rivers and lakes could come. What the origin of Ariid catfishes might be elsewhere would not be relevant to the origin of Luzon Ariids. *Arius magatensis,* living in a mountain valley at an elevation of 1,000 to 1,200 feet above the sea, could only have arrived from the sea.

Other rovers which have left the sea and ascended the Cagayan River valley and are now permanent dwellers in the Magat Valley, Nueva Vizcaya Province, are a Phallostethid, *Gulaphallus eximius,* and a halfbeak, *Zenarchopterus philippinus* var. *magatensis.*

One of the most surprising visitors to fresh water is the little stinging sea-wasp, *Gymnapistes niger.* It has been taken in the Zambales mountains 42 kilometers from the sea, as well as in river rapids near the sea in Mindanao. The family Scorpaenidæ, to which it belongs, is a group of reef dwellers, and one the reefs is where we usually find the black sea-wasp.

Some kinds of fishes which go to the sea to spawn never return. Best known of these are the fresh-water eels of the genus *Anguilla.* The gigantic females of *Anguilla marmorata,* known as "pabukangbinhi" in Tagalog, may be two meters long with a circumference of 450 mm when they leave their inland home after some years and go to their unknown breeding ground, there to spawn and die.

Here I shall drag in an irrelevant fact concerning *Anguilla pacifica,* an item omitted from my account of Philippine eels. When alive or in a fresh condition, *Anguilla pacifica* has bright orange nasal tentacles, a conspicuous character not mentioned by any author.

Other fishes such as bañgos (*Chanos chanos*), gingao (*Lutianus argentimaculatus*), the mullets (family Mugilidæ), and the various species of *Caranx* never return to fresh water when they once leave it. As they do not die after spawning one might suppose that some of them would go back to the streams or lakes for another season of rich living, but there is no evidence that they ever do so.

Among the gobies whose young enter fresh water and remain there until nearly ready to spawn, the mortality is very great among the fry ascending streams. It is evident that there is also considerable mortality among those returning to the sea or river mouth to spawn, especially where they descend rocky and turbulent hill streams. It is equally evident that those which survive the perils of spawning and the hordes of enemies encountered in their long journey likewise ascend the rivers again and return to their highland abodes. This is well shown by the fact that now and then we find in some upland valley or other interior point an eleotrid, such as an Ophiocara, or a goby, as one of the species of *Glossogobius*, which is very bulky and four times as long as the size at which the species begins to breed. There is no direct evidence as to what age these fish may live nor how many times they may make the round trip from sea to the interior and return.

Various eleotrids and gobies were kept in fresh-water tanks in Manila for periods ranging from three to about ten years, and all were still vigorous at the end of the time. There is accordingly no question about the natural span of life being amply long enough to make a number of round trips. But the perils of the journey and the hazards from enemies are so great that the number of fish making more than one or two round trips must be very small.

The largest fish entering Philippine fresh waters is the tagan or sawfish, *Pristis cuspidatus*. In 1870, Dr. A. B. Meyer, a celebrated Geman naturalist, spent a month at Santa Cruz, Laguna Province. He reported that every day several large sawfish, up to a length of 20 feet, were brought to the Santa Cruz market. Later, in Rizal's time, he told in his noted novel, "The Social Cancer" of huge sawfish being caught in fish corrals near Calamba. Nowadays the Pasig River and Laguna de Bay are too full of ships and motor launches for any but very small sawfish to enter. During the last twenty five years I have seen no sawfish from Laguna de Bay more than a meter in total length, and even those were quite rare. The Rio Grande de Mindanao or Pulangi, and the vast Liguasan swamp were still frequented by sawfish four to five meters long when I used to visit those regions. But no doubt the great increase in population and water traffic now prevent large sawfish from frequenting those waters.

Another large fish occurring in rivers and lakes in many parts of the Philippines is the Ganges pating or Ganges shark,

Carcharias gangeticus. It enters all the rivers of Mindanao except those too small or too steep, and ascends the Agusan to Monkayo and beyond. I found the Ganges shark and the sawfish well known along all the rivers of Cotabato and Davao provinces. The Monobos and Mandayas along the upper Agusan had the curious belief that the sawfish was the male, the shark the female of the same species. The Ganges shark lives in both Laguna Bombon and in Lake Naujan. Once when cruising in a small canoe along the west shore of lake Naujan a Ganges shark about three meters long was observed chasing a gray snapper or *aliso* (*Lutianus argentimaculatus*). The water was very shallow and the shark was practically half out of it. Just as the shark was about to catch the snapper, which weighed six or eight kilos, the *aliso* gave a mighty leap into the air and landed in a dense bed of reeds growing in still shallower water, so shallow that the shark could not force his way further.

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FISHES OF THE RED SEA AND SOUTHERN ARABIA

By Henry W. Fowler *

A REVIEW

By Albert W. C. T. Herre
School of Fisheries, University of Washington

The Weizmann Science Press of Jerusalem, Israel, has issued in attractive format in volume I of the "Fishes of the Red Sea and Southern Arabia." It includes all the fishes known from the area included, from the lancelets or Branchiostomidæ to the threadfins or Polynemida.

At first glance the reader may wonder what the fishes of the Red Sea have to do with the Philippines. As a matter of fact, there is a very real and vital connection between the marine fishes of the Philippines and those of the Red Sea.

The first writer to make any of the fishes of the vast Indo-Pacific known to Europeans was the Swedish naturalist and disciple of Linnaeus, P. Forskål, who published the results of his studies in 1775. In 1828, E. Rüppell published his "Fishes of the Red Sea," and in 1835 appeared his second monumental work on Red Sea fishes.

As scientists extended their collecting eastward into the East Indies, Philippines, and Polynesia, they found to their amazement that a surprising number of the fishes described by Forskål or Rüppell also occurred in these waters so far away. It is for this reason that the present work is of interest to Philippine scientists. Anything treating of Red Sea fishes helps throw light upon the distribution of Philippine fishes. Really the greatest center of marine fish life in the world is the Philippine, East Indian maze of tropical waters, and the Red Sea is actually its western fringe. But the Red Sea is important as it was the first area from which this vast fish fauna was made known to science.

Fowler's work under discussion is not based upon large new collections, but is in part compiled from the literature and in part on studies of available specimens.

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A synopsis of the classes is given, followed by descriptions of the orders, suborders, and families. The genera are not described, but keys are given to them where needed. When there is more than one species in a genus, a key is provided, and there is a brief description of each species. The distribution of each species is given, either with the synonymn or at the end of the description. There are many illustrations in outline, drawn by the author. Some are copies from other authors. The synonymy is given only when included within the region covered by this work. Vernacular names are given where known.

The classification used is in general that of the author's valuable "Marine Fishes of West Africa," (Bulletin, American Museum of National History, New York, vol. 70) but modified and condensed.

This is a useful work in that it brings together the available information about the fishes of an imperfectly known region and emphasizes their distribution. This information is widely scattered, often in works scarcely available nowadays. It is hoped that the second volume may be made available soon.

On page 196 in the bottom paragraph is a curious error. The author is speaking of pipe fishes and says, "They are poor swimmers, moving about in vertical position by undulating movements of the dorsal fin." He is evidently referring to the sea horses, *Hippocampus* species. Having kept many pipe fish and sea horses in acquaria over a period of years, I can safely assert that pipe fish do not swim in a vertical position, but that sea horses do.

BOOKS

Books received from time to time by the Philippine Journal of Science are reviewed and acknowledged in this section.

Transmission Circuits. By Eduard Williams and James Woodford, Jr. New York, The Macmillan Company, 1957. 156 pp., 76 figs. Price, $4.25.

This book, written by two professors from the Carnegie Institute of Technology, is intended as a standard text for senior students in electrical engineering. Its scope covers the fundamental principles of power and signal transmissions and the methods whereby they are made applicable to practical engineering problems. The opening chapter deals on the theory of the generalized transmission circuits. Students who are familiar with the hyperbolic functions and differential equations will appreciate the formal derivation of the relation of the voltage and the current at the sending end of the transmission line with that at the receiving end. The second chapter is devoted in the discussion of the theory involved in the determination of the transmission line parameters and the skin-effect. The discussion about the skin-effect is fairly thorough but requires a good understanding of differential equations and electromagnetic field theory which undergraduate students are hardly expected to possess. However, this part can be skipped without affecting the study of the subsequent chapters.

In the analysis of power transmission line problems, the authors introduced the four-terminal equivalent network, which can be either the "Pi" or the "T" configuration, to simulate the actual conditions. The network composed of bilateral elements relates the voltage and the current at the receiving end to that at the sending end by the general formula.

$$E_S = AE_R + BI_R$$
$$I_S = CE_R - DI_R$$

in which the coefficients A, B, C, and D are functions of the line parameters and of the length and frequency of operation. From these formulas evolved the sending- and receiving-end circle

diagrams which facilitate easy solutions to common transmission line problems. Aside from this method the author presented another approach to the solution by using the series expansion of the hyperbolic functions sinhp and coshp, which terms appear in the general transmission line equations.

Corrective measures to minimize the attenuation, distortion and crosstalk in signal transmission are well treated. In high frequency power transmission, the authors treat those problems dealing on transmission line structures, circuit behavior of the line and voltage wave distribution. The chapter on transients, where most other texts employ advanced mathematics, is much simplified by using the simplest approximate solution applied to transmission line of negligible losses. Partial differential equations are nevertheless involved. Of practical importance that more than justify their inclusion in a condensed text like this are the location of signal line fault by using a.c. bridge and the method of impedance measurement at frequencies above those at which the conventional bridges are useful.

At the end of each chapter there are useful problems for the student to work out. As a whole, this is an excellent text which may be recommended for use in a one-semester course.—G.R.J.

PUBLICATIONS AVAILABLE

CONTENTS AND INDEX. THE PHILIPPINE JOURNAL OF SCIENCE, vol. 1 (1906) to vol. 10 (1915). Bureau of Science Publication No. 8 (1917). Paper, 442 pages. Price $2.00 United States currency, postage extra.

SECOND TEN-YEAR INDEX. THE PHILIPPINE JOURNAL OF SCIENCE, vol. 11 (1916) to vol. 28 (1925). Compiled by Winifred I. Kelley. Bureau of Science Monograph 26. Paper, 382 pages. Price, $2.00 United States currency, postage extra.

CHECKLIST OF THE ANTS (HYMENOPTERA: FORMICIDÆ) OF ASIA. By J. W. Chapman and S. R. Capco. Institute of Science and Technology Monograph 1 (1951) new series. Paper, 327 pages. Price, P2.00 United States currency, postage extra.

NOTES ON PHILIPPINES MOSQUITOES, XVI. GENUS TRIPTEROIDES. By F. E. Baisas and Adela Ubaldo-Pagayon. Institute of Science and Technology Monograph 2 (1952) new series. Paper, 198 pages with 23 plates and 4 text figures. Price, $2.50 United States currency, postage extra.

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